Volume 22 Number 6

JOURNAL of DENTAL RESEARCH

FOUNDED BY WILLIAM J. GIES IN 1919

Official Publication of the

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

December, 1943

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PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORIAL OFFICE: 4559 Scott Ave., St. Louis, Mo

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Subscription price $5.00 per volume. Single numbers when available $1.00.

Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 187

WAVERLY PRESS, INC.

BALTIMORE, U. S. A.

IN VITRO PRODUCTION OF PLAQUES AND CARIES[1]

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Magitot (1), Miller (2) and other early investigators, as well as numerous investigators of the twentieth century, have been successful in their attempts to decalcify teeth. Extracted teeth were used for this purpose and introduced, among other variables, the condition of non-vitality. However, as early as 1897 Tomes (3) concluded that vital forces played no part, and that caries was the effect of external causes. Wallace (4), among the present-day investigators, maintains that "dental caries is the result of a chemico-parasitic process originating outside, and independently, of the substance of the tooth".

Most recently, Berggren (5), antithetically, has demonstrated by the penetration of dyes, *intra vitam*, that there exists a communication between enamel and dentin. This investigator, however, "is quite aware of the fact that permeability may be looked upon as a simple diffusion without vital influence. However, it is not probable that staining would have been so successful by physical means alone, and the difference of the staining *in vivo* and *in vitro* indicates that conditions in the vital tooth differ from those in the extracted tooth".

There are certain imperative criteria, in addition to non-vitality factors, characteristic of natural caries which, heretofore, have not been reproduced *in vivo*. These features, found coexistent with natural caries, should not be ignored when attempting to produce artificial caries. Among these factors are: 1. The entire tooth should be exposed to the saliva and the lesion should result only at a predetermined site in the enamel to which a hydrolyzable carbohydrate substrate is added. 2. The total salivary acidity must not be such as to produce decalcification readily. 3. Saliva must be as fresh as possible, from susceptible subjects *and in motion*.

It is definitely known that the lesions occur in areas where detritus is retained upon the teeth, and, in contradistinction, it rarely occurs upon the cleansible areas which are scoured by masticatory action. The dirty mouth and rampant caries are not necessarily coexistent. However, it appears that *sine qua non* to the caries process, are the presence of acidifiable foodstuffs and acidogenic bacteria.

APPARATUS (MCG)

An apparatus was designed to circumvent some of the ordinary difficulties in the production of artificial caries. It may be observed (*fig. 1*) that the total

Revised by author October 5, 1943.

[1] Received for publication June 9, 1943.

[2] Now at Army Dental School, Army Medical Center, Washington, 12, D. C.

apparatus is built around the microscope which permits the direct observation of the "living" lesion. The ordinary method of producing artificial caries in a

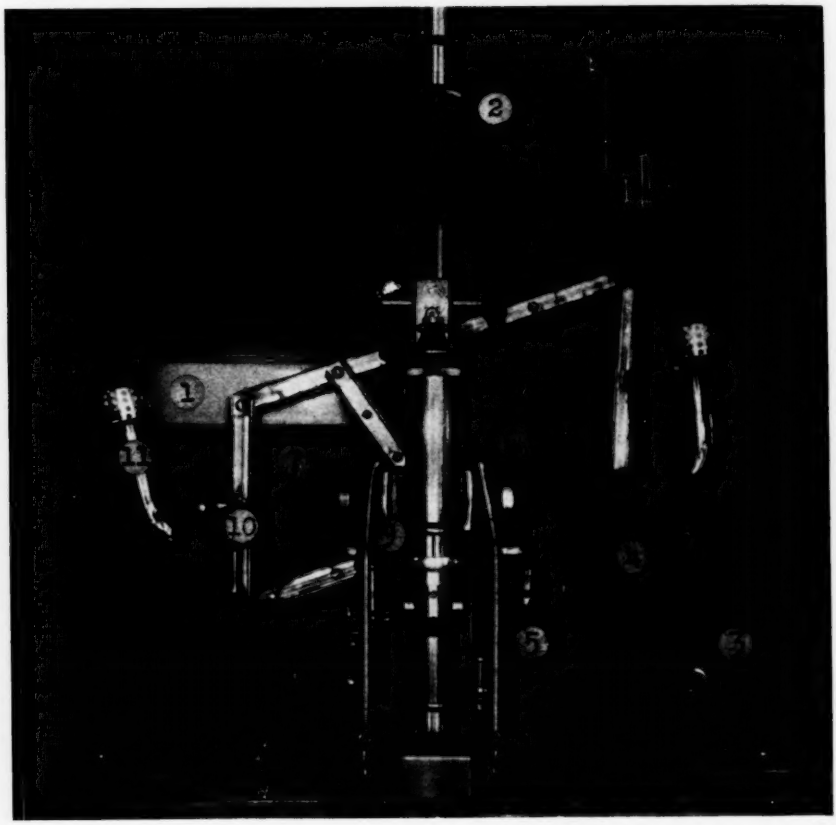

FIG. 1 shows the entire apparatus placed in a modified Dekotinsky incinerator. The system is maintained at 37°C. to assure optimal bacterial multiplication and enzymatic activity. 1 is canopy for vacuum-controlled dissipation of excessive heat radiated from motor and light; 2—thermometer (obscured); 3—master switch; 4—chronometer, electric clock motor, operates larger electric motor (7). This causes saliva to be set in motion for 15–20 secs. every hour; 5—chronometer light permits observation of revolving armature through glass pane to facilitate observation of armature assuring its operation as clock motor is not bichronous; 6—gear system is tri-ratioed to produce mechanical advantage of power and preferred rate of "teeter-tottering" oscillations of frame (6 complete oscillations in 20 secs.); 7—electric motor operates oscillating frame (8) and is governed in its on/off phases by chronometer (4); 8—oscillating frame; 9—specimen chamber in which tooth section is placed; 10—pure gum rubber tube; 11—glass L-tubes which, in conjunction with 9 and 10, contain total 50 c.c. of saliva used.

whole tooth and then making a ground section of it is reversed; a ground section is prepared, and then the lesion is produced in it. The specimen chamber is rigidly fixed to the stage of the microscope (*fig. 2*).

METHOD

Ground section. Enright, Friesell, and Trescher (6) found it necessary to use the labial surfaces of teeth rather than the approximal on producing artificial caries, because of the questionable structural integrity of the latter surfaces. That this is essential from a structural, and preferable from a methodological standpoint is quite evident.

After observing over 50 tooth-sections under various experimental conditions it was deemed advisable to polish the surface of the tooth from which the section was to be made. The observation of a "wet section" frequently revealed that the exteriormost 5–15% of the enamel is relatively amorphous in comparison with the well-defined structure of the remainder of the enamel. Box (7), in referring to this cortical enamel, cites Miller as saying, "Whenever the crust,

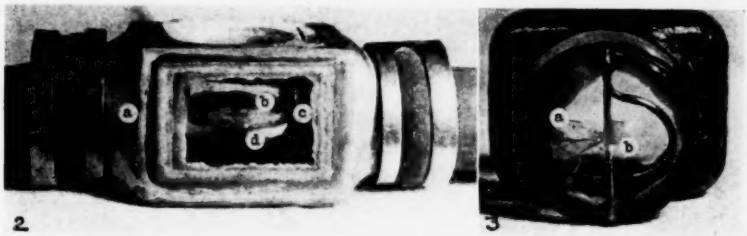

FIG. 2 shows the specimen chamber. (a)—specimen chamber which has windows. (One above to receive escutcheon to which tooth-section is clasped, one below for transmission of light and side windows to render visible coverslip and facilitate resolution.) Tubulatures at either end serve as attachments for rubber tubes. Silico-aluminum chamber is completely paraffinized on internal surfaces to preclude any oligodynamic effect. (b) section of glass tube is telescoped into specimen chamber so that coverslip is about 5 mm. above surface level of saliva during quiescent phase. (c)—round coverslip is fixed to tube with inert, adhesive, and saliva-resistant wax. (d)—long axis of periphery of enamel of section is placed perpendicular to kinematic path of saliva. Resilient gold alloy clasp securely holds the section against the coverslip under a constant spring tension (fig. 3).

FIG. 3. Escutcheon removed from specimen chamber and viewed from beneath. Clasp may be seen securing ground section to coverslip with tines of capillary feeding device directed perpendicularly to, and in contact with, enamel.

which is only a fraction of a millimeter thick is mechanically removed, the acid at once acts far more rapidly upon the enamel than otherwise".

Because the method herein described deals with the microscopic changes in the enamel as seen in incipient caries, the minute surface irregularities within the frequently encountered cortical enamel were eliminated by polishing the surface of the tooth before sectioning it. In no instance was all of the cortical enamel removed; in fact, just enough was removed to render the surface absolutely smooth.

The tooth-section is quasi-histological and some few modifications are contingent upon the technical factors involved. The section is ground to a 6° wedge or plano-sphenoid section. The 6° plano-sphenoid section is ground to 100 microns in thickness at the enamel periphery which diverges to thrice that thickness in the dentin.

The section after completion is treated with a 5% lactic acid for a few seconds and is immediately rinsed with distilled water to rid the surface of abrasive dust and improve its histological clarity. The predetermined zone of the lesion is centrically placed on a $\frac{7}{8}$ inch circular coverslip and held under spring tension by a noble metal clasp.

Pabulum. The white bread used in the MCG was macerated and autoclaved which rendered it papescent and materia alba-like in character. Various pure carbohydrates were used less successfully. Other than the 50% of hydrolyzable substance, bread contains sufficient insoluble substance to act as a matrix for a duration of approximately 6 hours. At point (a) in *fig. 3* a pin-head size piece of pabulum is inserted with a dissecting needle. The pabulum is actually in contact with only 0.10 mm. of the peripheral enamel with a portion of it contiguous to the coverslip. The condition represents the caries mechanism on a natural scale. The pabulum is replaced every 8 hours after gently rinsing the section on the coverslip with distilled water.

Lesions could not be produced in tooth sections unless the pabulum were in absolute contact with the enamel periphery because of the diluent and buffer capacity of the organic and inorganic substances within the saliva. The pabulum was added to the surface of the tooth section in conformity with those conditions permitting the residence of food debris *in vivo*. A considerably greater area of the tooth-section was exposed to the saliva than that to which the carbohydrate is added. In consequence of which only the site to which the pabulum is added and not the immediate adjacent areas will decalcify. In this way the tooth-section, *in toto*, will act as its own control against extravagant decalcification.

Saliva and salivary tests. The conduit system consisting of the specimen chamber, rubber tubes, and glass L-tubes are filled with 50 c.c. of gauze-filtered saliva taken from 2 caries-susceptible subjects and mixed. Fresh specimens of the saliva were replaced every 24 hours and the entire system thoroughly washed with soap and water before replacement. Distilled water was allowed to trickle gently over the section and fresh pabulum again added at this time. To the saliva, 120 mgm. of glucose were added to assure a pH potential of approximately 6.0. Occasionally supplemental amounts of glucose or carbamide (synthetic urea) were added to maintain this pH value. This required half-hourly potentiometric tests, for it was found that the pH potential for any sample of saliva was wholly unpredictable.

In order to maintain the most desirable salivary consistency the MCG was constructed, and so regulated, that it would agitate the saliva only enough to uniformly distribute all the salivary elements.

Saliva acts constantly, although to varying degrees, as a diluent factor in the oral cavity. Wallace (8) regarded a copious flow of saliva caused by acids, and pungent bitter principles, as the "natural mouthwash that should be stimulated". Macphee (9) mentioned that "if much saliva is secreted it is always being swallowed, and replaced by fresh saliva, so that the environment of the teeth is constantly changing, and stagnation is less likely to occur". However, the process is not quite so simple as all this. Freshly secreted saliva does not

replace the saliva which is present, but by partial displacement attenuates saliva held *in situ* on the teeth by surface tension, gravity, capillarity and other physical means. The saliva, therefore, is an effective diluent only in direct proportion to its ability to insinuate itself into the numerous crevices of the soft structures and interstices between the teeth. Hence, the diluent-displacement action of the saliva is only a mild detergent factor which is not equally effective at all times. Saliva is also detergent as a vehicle of diastase. It is well known that there are times when an individual demonstrates copious salivation, and on other occasions during the same day may present a mouth so devoid of saliva to be suggestive of xerostomia. In view of the foregoing conditions and the extremely gentle diluent-displacement action of the saliva in the mouth it was thought best to agitate it intermittently only in the MCG. Therefore, the MCG was designed to have a dynamic phase alternating with a quiescent phase.

TABLE I

STATIC		DYNAMIC	
Hrs.	No.	No.	Phases*
0	56	60	Pre-dynamic
1	44	52	Post-dynamic
2	41	30	Mid-quiescent
3	30	11	Pre-dynamic
4	17	10	Pre-dynamic
5	8	62	Mid-dynamic
6	2	66	Post-dynamic
7	0	18	Mid-quiescent
8	0	58	Pre-dynamic

* Fluctuations in the readings of the dynamic saliva are due to the time within the dynamic/quiescent cycle when the specimen is withdrawn.

Benedict and Kanthak (10) stated that enamel would dissolve in the saliva were it not for the calcium and phosphorus ions. This effect was apparently borne out by Volker (11), who has demonstrated, radiographically, that animals fed radioactive phosphorus show some absorption of this element on the exterior of the tooth. In this case the buffering effect seemed to be, essentially, one of phosphatization. The natural buffering capacity of the saliva is employed in the method under description.

Teeth in the mouth are usually kept moist or wet by just a film of saliva. For this reason the tube of the escutcheon was telescoped into the specimen chamber only so far as necessary in order to have the section submerged only during the dynamic phase, and to retain just a "film" of saliva during the quiescent phase, during which time the total saliva recedes from the section. Previously it had been found impossible to initiate caries if the section were constantly submerged in the saliva.

Whereas, in this investigation, it was desired to limit the pH of the total saliva to neutrality, it was found that caries cannot be initiated unless it has been de-

pressed to an average pH of 6. With the total saliva held at an average pH of 7.0 there is noticed excessive turbidity, and a putrefactive odor which is even imparted to the rubber tubes of the conduit system.

Preliminary tests were designed to prove the uniformity of the salivary elements. 120 c.c. of thoroughly agitated paraffin-stimulated saliva was divided into 2 parts, 60 c.c. was placed in the MCG and 60 c.c. in the incubator in a graduate. Six specimens were pipetted from the various levels in less than 4 minutes. One drop from each level was placed on a slide (2 being used) and divided into 3 sections. These were then fixed and Gram-stained. The number of epithelial cells in each drop of each specimen from the various levels was counted. The epithelial cells in 60 c.c. of saliva thoroughly shaken in a graduate, gave in 5 samples of saliva (50 counts) the composite seen in *fig. 4*. This observation indicates that the number of epithelial cells, irrespective of type (12), are equally distributed throughout saliva in motion. To demonstrate the waning stability of this rather uniform distribution, 60 c.c. of paraffin-stimulated saliva was placed in a graduate and thoroughly shaken, and a drop pipetted from the midlevel (30 c.c. level) every hour during standing in an incubator at 37°C. (static condition). A composite of 5 specimens gave the results as seen in *fig. 5* This remarkably consistent sedimentation rate of epithelial cells indicates the vast differ-

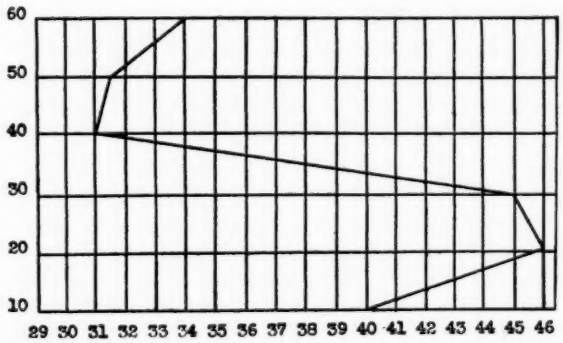

FIG. 4. Uniformity of epithelial cells. Abscissa—number of cells; ordinate—level from which saliva was secured.

ence between static and dynamic saliva. The epithelial cells having the greatest density of the total salivary elements afforded a simple index for the determination of the minimum amount of agitation necessary to uniformize the saliva.

To determine how much of an effect other elements in the saliva might have on the epithelial cell sedimentation rate 60 c.c. of paraffin-stimulated saliva was filtered through a fine grade of filter paper and liberally washed with distilled water until virtually bacteria-free. It was roughly estimated that about half of the epithelial cells passed through the filter paper as evidenced by the number appearing in the filtrate. Therefore, to keep the ratio equal between the epithelial cells in dispersion, and the dispersion medium (distilled water in this case), the residue was added to only 30 c.c. of distilled water. The graduate was stoppered and thoroughly shaken. This distribution of epithelial cells (*fig. 6*) was found to be of greater uniformity than when dispersed in saliva. The difference lies in surface tension and the coefficient of viscosity, and stimulated saliva is invariably far less viscous than unstimulated. In this procedure the same technique of counting the epithelial cells applies. After these tests 20 c.c. of each sample of saliva (3) was decanted into a graduate to make 60 c.c. This was then thoroughly shaken and kept under static conditions for the remainder of the experiment. A reading was made every 15 minutes by pipetting from the mid-level (30 c.c. level) and employing the same technique (*fig. 7*).

The foregoing was interpreted as indicative of the fact that the epithelial cell sedimentation rate is determined by elements within the saliva other than the epithelial cells themselves. The epithelial cell sedimentation rate, therefore, is a measure of the gravity factor (density) of the epithelial cells against the bouyancy factors (densities) of all other elements in the saliva. That surface tension factors play at least a subordinate role is not doubted.

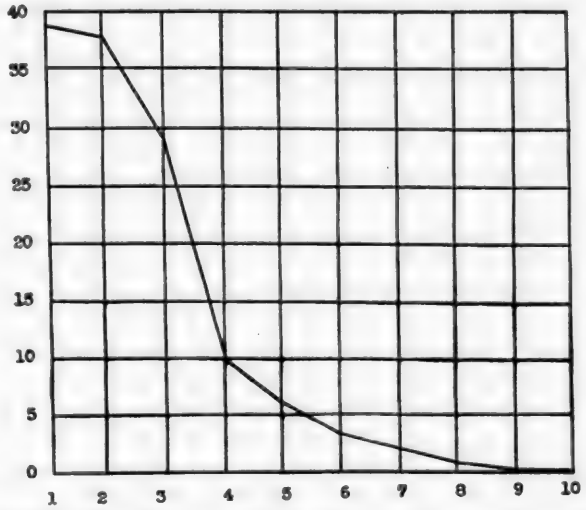

FIG. 5. Epithelial cell sedimentation rate. Abscissa—hours; ordinate—number of cells from mid-level.

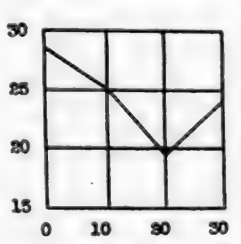

FIG. 6

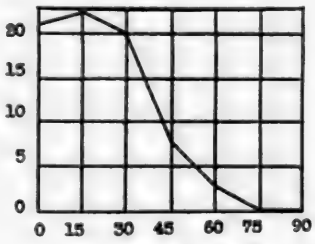

FIG. 7

FIG. 6. Distribution of epithelial cells in distilled water. Abscissa—level; ordinate—number of cells.

FIG. 7. Epithelial cell sedimentation rate under static conditions. Abscissa—minutes; ordinate—number of cells.

From the foregoing experiments the epithelial cells are thought to constitute a fairly reliable index of the uniformity of other constituents in the saliva.

In the next step a comparison was made between the epithelial cell sedimentation rate of static saliva with saliva in the MCG under dynamic conditions. For this experiment 120 c.c. of paraffin-stimulated saliva was placed in a graduate and thoroughly shaken. Sixty c.c. of this saliva was poured into a graduate and placed in the incubator (static condition), and the remaining 60 c.c. was placed in the MCG (dynamic condition). A few drops were

pipetted from the surface of the saliva from the specimen chamber of the MCG and from the mid-level of the graduate in the incubator. In the specimen chamber the specimens of saliva were pipetted from immediately beneath the surface because this level of the saliva will contact the tooth-section in the specimen chamber. The specimens were taken hourly and the technique of counting epithelial cells as previously mentioned applies. A comparison of saliva under dynamic and static conditions using the epithelial cell sedimentation rate may be seen in Table I. All quantities of saliva given in this paper refer to the quantities read at the fluid level of the saliva disregarding the supernatant froth.

The results indicate that the MCG does assure a far greater uniformity of the salivary elements, and that saliva in motion is certainly unlike static saliva. The purpose of undertaking the study of caries produced artificially with "dynamic" saliva is intended to exert the following effects on the tooth-section: 1. To supply the pabulum with uniform salivary elements. 2. To supply the pabulum with sufficient diastase. (This initiates the degradation of the pabulum, permitting the bacterial enzymes to act upon the simpler carbohydrates.) 3. To exert the effects of the total bacterial flora upon the local flora. 4. To faithfully reproduce the oral environment relative to the ablatitious and buffering action of the saliva.

Some quantity of glucose must be added to the saliva so the bacteria are able to hydrolyze the addendum, ultimately, to acid products. Preliminary experiments indicated that 120 mgm. of glucose in 50 c.c. of saliva will not permit the saliva to become excessively alkaline. Control tests gave evidence that 5–15% less glucose need be added under dynamic conditions than static. This follows the observation of Bibby and MacKinnon (13).

The removal of particulate matter from the saliva by filtering it through 6 thicknesses of fine surgical gauze, which had been saturated with distilled water to remove the lint, only slightly affects the saliva. After filtering 25 c.c. of a thoroughly shaken 50 c.c. specimen of unstimulated saliva, to which 120 mgms. of glucose had been added, it was found upon comparison of the filtrate with the original that there was greater clarity and no froth in the former. That this physical refinement does not appreciably alter the viable bacterial count was determined by the viable count plating method. This experiment was performed with four 50 c.c. specimens of caries-susceptible saliva from different individuals. Immediately after collection each specimen of unstimulated saliva had added to it 120 mgms. of glucose and is thoroughly shaken. From each specimen 0.5 c.c. was spread over blood agar plates before and after filtration. After 36 hours the colonies are counted on the 4 plates to which had been added the filtered saliva and on the 4 plates to which the unfiltered saliva had been added. The ratio of colony growth was found to be 7 unfiltered to 5 filtered. The 4 filtered specimens are mixed together, also the 4 unfiltered specimens and placed in separate graduates. The specimens were found to have pH values of 8.2 and 7.9 respectively. This reading was made after 4 hours of incubation at 37°C. These alkaline values necessitated adding supplemental amounts of glucose to each specimen in order to have sufficient acidifiable substance to permit the determination of the acid potential. An arbitrary supplemental amount of 100 mgms. of glucose was added to each 100 c.c. specimen. The 2 specimens were incubated and the pH values which were made hourly may be seen in *fig. 8*. That the slight difference in the final pH may have been due to the concentration of bacteria follows the statement that, "the important factor appears to be the ratio of the number of bacteria to the volume of the fluid medium . . . assuming that the organisms are evenly dispersed throughout the medium" (14). However, the removal of residual carbohydrate substances from the saliva may have some slight effect.

The natural alkaline potential of saliva increases almost 0.5 of a pH unit for each hour during the first hour or 2 of collection. A diphasic curve was found for a composite of 5 salivas with the pH of the first 5 c.c. of freshly secreted saliva being about 7.5. To a 50 c.c. aliquot of the saliva (collected in 1 hour) 120 mgms. of glucose was added. At the second hour, i.e., the time at which glucose was added, the saliva tested pH 7.7 and decreased to pH 7.5 at the third hour after which the acidic phase became dominant.

Specimens of saliva under conditions resulting in a profound diphasic curve invariably liberate an unmistakable putrescent odor although testing decidedly acid. After realizing the inordinate variability of the saliva it was thought to be of importance as to what hour after adding glucose that it is completely exhausted. For this experiment 5 c.c. of unstimulated saliva was taken from 15 individuals to make 75 c.c. To this was added a comparable "threshold" amount of glucose (180 mgms.) and the material was placed in the MCG under dynamic conditions. The pH of the saliva was determined every hour and Benedict's qualitative test for sugar made. This was positive at the first hour (pH 7.0), positive at the second hour (pH 7.5), plus minus at the third hour (pH 7.3) and negative at the fourth pH 7.0), fifth (pH 7.4), and sixth (pH 7.4) hours.

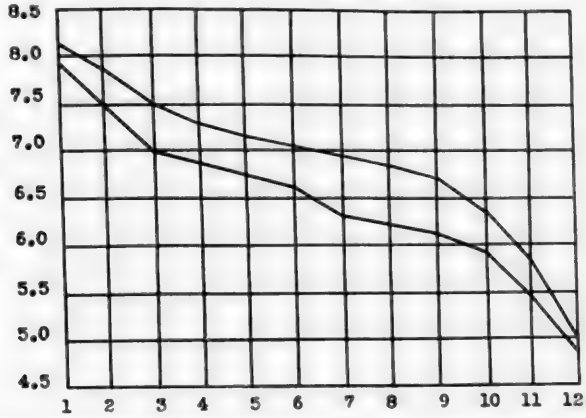

FIG. 8. Relative parallel course in decrease of pH values in filtered (upper line) and unfiltered (lower line) saliva. Abscissa—hours; ordinate—pH.

In subsequent tests it was not found necessary to make concomitant sugar determinations. Instead, whenever sharp troughs appear in the curve, the saliva is fortified with additional glucose and is tested half-hourly thereafter. This forestalls an excessive alkaline phase and alters the vector of the curve. Occasionally it is found that the "threshold" amount of glucose is too great for a certain specimen of saliva. In this instance, with half-hourly pH determinations made and no diphasic curve occurring, a sharp decrement towards acidity is frequently indicative of an unusually high acid potential. In order to avert the occurrence of an excessive acid phase a controlling quantity of a saturated carbamide solution (synthetic urea) was added. Carbamide, as maintained by Stephan (15), is hydrolyzed to ammonium carbonate by several strains of organisms indigenous to the oral cavity. It has been found that when adding carbamide or supplemental glucose to preclude excessive acidity or alkalinity that, if smaller quantities of these substances are added oftener and potentiometric tests made at 15 minute intervals, the excessive fluctuations can be accurately controlled. If the salivary pH is stabilized to an approximate value of 6.0 within the first 6 hours there is but little fluctuation in the succeeding 18 hours after which time it is changed. That the stationary phase in bacterial multiplication has been reached by the sixth hour is quite likely. Bail (16) concluded that there is a limiting bac-

FIG. 9. Photomicrographs (*figs.* 9–12) were made through an interval of 1 to 2 cm. of saliva. Under these conditions it was necessary that the iris diaphragm be opened wide, losing to a considerable degree the contrast factor. Lesion has invaded to almost ½ thickness of enamel (about 0.6 mm.). Thickness of enamel at this point was about 1.5 mm. before the onset of lesion. Single flame from lesion proper projects to within 0.4 mm. of dentino-enamel junction. At end of flame-like projection and dentino-enamel junction an artifact appears (bubble in saliva). Upon periphery of enamel, and immediately over lesion, thin velum-like bacterial plaque may be observed. In upper right corner there

terial population or density that could not be exceeded. Also that, the addition of an enriching substance, such as glucose, to the broth (in this experiment, saliva) increases the total number of bacteria produced, but the maximum concentration of viable organisms remains unchanged. That the polymicrobic flora of the saliva will show far greater variation in this respect than would any one pure strain of organisms, as used by Bail, is to be expected.

Catadioptric effects. It is thought best to explain the microscopic system of light used, as a physical component, such as the light intensity, is of importance insofar as it definitely influences the appearance of a wet section. The concave substage reflecting mirror is used to minimize the diffraction of light through the saliva. The interval of saliva is an integral part of the optical system, as is anything which interposes the optical axis. A photometer inverted over the specimen chamber permits of turbidimetric determinations. The photometric method as used by Mestre (17) is used only to determine the diminishing transmission of light which is compensated by opening the iris diaphragm of the Abbe condenser completely. The transmission of light is computed in foot candle power (f.c.p.). Transmission of 60 f.c.p. is adjusted by the use of the diaphragm. Sixty f.c.p. is used as the arbitrary standard on the basis that it appears to be the optimum light intensity for 27 tooth-sections previously observed.

The transmission factor is easily determined by using the fixed f.c.p. value (60) as the denominator. Hence, 30/60 = 50% (transmission factor). In this fraction, with 30 representing the transmission intensity, the equivalent 50% (transmission factor) indicates that the light intensity within the optical system has been reduced, as is true in the average case, to 50% of its original transmission intensity by the saliva. Using a graph computed for the apparatus the turbidity for any hour may be determined. There is a decided increase (53%) in turbidity during the first hour, after which time there is a striking negative increase. The turibidity is 50% greater from the 10th to the 12th hour than at the time that the saliva is just decanted into the conduit system. The physical nature of the agglomerates, although numerically greater, are smaller under dynamic conditions than static.

Bacterial plaque. Black (18) and Williams (19) demonstrated that gelatinous plaques, containing "caries fungi" might be a possible factor in the production of the carious lesion. Lothrop and Gies (20) regarded plaques as composed of mucin or its alkaline salts, and believed that mucin may favor the onset of caries by facilitating the adhesion of bacteria to the tooth surface. However, later Lothrop (21) showed that acid in concentrations capable of dissolving enamel also precipitates salivary mucin in a flocculent non-adhesive form, and will thus

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appears flocculent plaque of filamentous organisms projecting perpendicularly into saliva. Although out of plane of focus a similar "secondary" plaque occurred about same distance cervical to lesion. Initial bacterial plaque and "leptotrichoid" plaque centripetalize in their proliferation over ultimate site of lesion proper. Lesion as seen required approximately 72 hours to be formed and surface continuity is only slightly affected. Black object in lower left corner is arm of clasp.

FIG. 10. Occurrence of V-shaped etchings into interprismatic substance. This area was also photographed after 72 hours and constitutes exterior of lesion proper (orig. mag. × 1500).

FIG. 11. Filamentous organisms showing vibratory movement and terminal attachment to enamel periphery. Hyphal segments of lower mycelium are in evidence. Other bacterial forms may be identified within saliva (orig. mag. × 1500).

FIG. 12. Same area as shown in *fig. 10* after 148 hours. Artifact traversing the enamel rods persists in both views and is a distinct aid in orientation. Within this cleft secondary "bays" may be seen; often with sprightly oscillating bacteria within these confines at the time that dissolution of enamel occurs (orig. mag. × 1500).

facilitate the removal of plaques rather than to abet their formation. Blayney, Kesel, and Wach (22) considered that, "some bacterial plaques do not produce caries" and have reported a method of distinguishing between carious and non-carious plaques by their bacterial content as seen in smears where organisms appeared that were regarded as lactobacilli. Rosebury (23) stated that, "it is impossible from these data to evaluate the status of the 'plaque' question. If it is a structure specifically associated with dental caries, we are as yet ignorant of its distinctive nature and can only speculate as to its importance."

Lactobacilli and streptococci are invariably recovered from the surface of the lesions and the former are present in large numbers as evidenced by the luxuriant growth of the organisms on tomato-agar plates to which highly diluted portions of the plaques are added. Yeasts, and staphylococci occasionally throve on the tomato agar. The only other media used were blood-agar and Brewer's media (sodium thioglycolate) which revealed most frequently small gram-positive rods, gram-negative diplococci, streptococci, and gram-positive bacilli. The filamentous organisms seen in direct smears, that are gram-stained, appear to be leptotrichoid, and oidium-like forms. The heterogeneity of bacterial forms other than lactobacilli and streptococci encountered did not permit of further cultural, morphological, and biochemical identification. Direct smears also gave evidence of numerous fragmented and pyknotic organisms which leads the author to believe that the degraded proteinaceous fractions of bacteria constitute to a considerable degree, at least by this method, the homogeneous substance of the plaques.

ARTIFICIAL CARIES

With the saliva adjusted to an average pH of 6.0 over a period of 24 hours, only the periphery of the enamel in the immediate vicinity of the pabulum showed a definite structural change of a caries-like nature. This "living" lesion was constantly observed under the low power of the microscope. With the pabulum replaced 3 times during the 24 hour period there was evidence at the end of this period of brownish decalcification (white macroscopically) with absolute surface continuity. Within 72 hours the lesion progressed as seen in *fig. 9*. At this time V-shaped etchings into the interprismatic substance may be seen, under high power, in *fig. 10*. Some time later the small V-shaped etchings merge to form larger ones with the loss of the intervening structure—the enamel rods. The zoöglea of bacteria constituting the plaque was adherent to the periphery of the enamel and was found to form in a centripetal fashion. Bacteria seen in direct smears made at various times from the plaque were found to be essentially yeasts, gram-positive rods, leptotrichia, gram-negative diplococci, streptococci, gram-positive bacilli, and filamentous organisms *fig. 11*. Although the experiment was conducted for 1008 hours (6 weeks) no further structural change could be effected beyond the cavity seen in *fig. 12* which occurred after 148 hours. The loss of surface structure is actually less than 0.6 mm. as seen under oil immersion.

DISCUSSION

This experiment was undertaken primarily as a teleological study of the pathogenesis of the carious lesions of teeth. The hope was entertained that a thorough knowledge of local phenomena might, by innuendo, point to important local mechanisms. This *in vitro* method was, therefore, conceived to be not an end in itself, but rather, a means to an end.

The question is: "Can a typical carious lesion be produced in non-vital teeth by exogenous agencies alone?" Nowhere in the literature could an experiment be found which dealt with the production of artificial carious lesions using saliva, the acidity of which is not in itself of decalcifying potency. It need not be stressed that saliva of decalcifying potency, relative to its general pH value, is not normally found in even the carious mouth. Lesions produced by resorting to highly acidified saliva are spurious not only in the method but also in the result. The lesions produced in highly acidified saliva are localized only by virtue of the fact that the entire tooth is covered with paraffin, or some such suitably inert substance, with the exception of a small opening which is the chosen site at which "caries" is obliged to strike. The lesions produced under such conditions are decidedly unlike caries, at least in the experience of the author.

The lesions produced in the MCG, are not impeccable, yet the results obtained thereby point to the fact that a mere consideration of dental caries as a saprophagus process does not suffice. It is apparently true that acidogenic bacteria and hydrolyzable carbohydrate substances are prerequisite to the incidence of caries but these are, perhaps, but 2 necessary elements in a very complex process. It appears that sufficient acid could only be produced from simple carbohydrates and the more complex amylaceous substances taken into the mouth.

The author has found that the more rapid rate of acid formation in the saliva of caries-susceptible individuals is essentially due to the greater number of bacteria and their species multiplicity rather than the alleged lower buffer capacity of the saliva of susceptibles. Corroborating this observation, "the other factor", says Fosdick (24), "governing the rate of acid formation, the enzyme system, in all probability is regulated by the bacterial flora of the mouth. It has fairly well been established that no single organism can furnish an optimum enzyme system. However, it is also quite obvious that certain combinations of organisms can furnish an optimum enzyme system".

In one preliminary experiment with a tooth section the pabulum was washed out of the capillary feeding device about every half hour, and fresh pabulum replaced immediately. During the intervals between this procedure the section was placed in the MCG. The first day the peripheral enamel remained unaffected but on the second day, a thin, essentially bacterial, plaque was observed on the periphery. Slight etching was observed only under this plaque and occurred ½ hour after the pabulum was added, then washed and observed. This indicates that a locally adherent bacterial plaque is primarily essential and when once established, carbohydrate subsequently added to it may decalcify the enamel within a half hour. This lends credence to the fact that the almost instantaneous

drop in pH observed by Stephan (25) is the acid concerned with the causation of dental caries. Stephan has observed a depression beyond the critical decalcifying pH level of plaques in situ as low as pH 4.0. Although the original plaque of food debris may be distributed over a considerable portion of the coronal surface of the tooth, discrete caries apparently occurs only because of the residual food being acted upon by an established bacterial plaque confined to a smaller area.

In the method employed by the author it was possible to produce what might be conservatively called a caries-like lesion to a degree considered a little better than incipient. The lesion had its inception, on the average, about 2 or 3 days after the experiment had been begun at which time viable bacteria could be observed in a thin zoöglea over the very site where the lesion occurred. Although the entire section of the tooth is exposed to the effects of the saliva, only that area which had been covered with a hydrolyzable carbohydrate and an established bacterial plaque became affected with a caries-like lesion. The fact that the remainder of the tooth-section was unaffected is evidence that the lesion is due to a local acidity factor and not total salivary acidity. The section itself, in this respect, acts as its own control.

The lesion could at no time be made to penetrate for a depth over 0.6 mm. despite the fact that the experiment had been conducted for a period of 1008 hours (6 weeks). Under the oil immersion objective the bacteria could be seen intimately attached to the surface of the enamel with evidence of minute V-shaped etchings into the interprismatic substance. Lactobacilli and affiliated bacteria were readily demonstrated to be in residence over the lesion. Although lactobacilli and other microorganisms were invariably demonstrable from day to day by streaks made from the lesions, and because carbohydrate substance had been added every day, the reason for not being able to perpetuate the lesion is inexplicable.

At the time when the incipient etching of the enamel occurred a number of filamentous organisms could be seen in a plexiform arrangement attached to the affected enamel and offering at least mechanical retention for smaller bacteria. These filamentous forms assume particularly a perpendicular relationship to the enamel. The appearance is quite similar to that observed by Williams (26) of which he mentions that "covering the surface where decay had commenced there was always to be seen a thick, feltlike mass of acid-forming organisms. This mass of fungi is so dense and adhesive as to make it highly improbable that the enamel is affected, except in rare or special instances, by any acid other than that which is being excreted (sic) by the bacteria at the very point where they are attached to the enamel. The thick glutinous mass of fungi also prevents the excreted acid from being washed away so it exerts full chemical power upon the calcific tissue".

Today, almost a half century after Williams' microscopic demonstration of plaques and caries, the method set forth in this investigation affords the opportunity to observe an active lesion in its progression. At times bacteria in Brownian movement are observed within the triangular confines formed by the minute

V-shaped surface etchings and the contiguous pabulum. The lesion certainly simulates caries but did not, in any one specimen, penetrate more than half the thickness of the enamel (cervical third) or about 0.5 to 0.6 mm. It is averred that the lesion is much more caries-like in its incipiency than later when the acid attack instigated by the bacteria on the surface is more diffuse. The early lesion is discrete and well localized and may be seen immediately beneath the most proliferated portion of the bacterial plaque.

There are certain features of the artificial lesions which in the author's opinion are unlike true dental caries other than those already mentioned. These are: (1) The plaque is not as tenacious as those found on the teeth in the mouth. However, plaques formed within an average pH range of 6.0 are more tenacious than those formed within the higher pH ranges which are of a flocculent, papescent character. In connection with this Matthews (27) maintained that many of the polysaccharides are quite stable in an alkaline solution. This may account for the pabulum with the admixture of bacteria becoming a flocculent plaque rather than undergoing dissolution by hydrolysis and ablation. (2) The plaques formed in the MCG frequently evidenced numerous yeasts by direct smear and cultures. However, a number of investigators have been unable to show any correlation between yeasts or yeast-like organisms in respect to dental caries. Knighton (28) found yeast-like organisms in only 36.9% of caries susceptible individuals. Hemmens, Blayney, and Harrison (29) have been able to demonstrate in 39 carious plaques that 4 were positive for yeasts and in 41 non-carious plaques that 3 were positive for yeasts. Therefore, it may be said that yeasts do not appear to be essential in the causation of natural caries. In the artificial lesions produced by the author, lactobacilli were always present. This is not to be unexpected as the saliva of highly caries-susceptible individuals was employed in all instances. (3) A third feature of artificiality is, of course, the fact that the section is non-vital. However, caries in pulpless teeth have been reported by numerous investigators. Bodecker (30) ascribed the appearance of artificial caries to the absence of dental lymph. (4) A fourth feature which is not characteristic of caries is the apparent absence of flame-like extensions into the enamel. However, in some lesions produced, only after a long period of time, there is some evidence of this occurring. *A priori*, it appears that lesions presenting flame-like extensions are those which are slowly formed. (5) A fifth feature unlike caries is the inability to produce a lesion which invades at least to the dentino-enamel junction in the absence of cleavage lines.

In the author's clinical and laboratory observations it is found that cleavage lines or cracks characterize the enamel of practically all teeth. As a causative factor in caries, structural inferiorities have been suspected by numerous investigators and it is of great interest that cleavage lines are much more numerous in pulpless teeth. Fish (31) was one of the first to demonstrate the lack of permeability in human enamel using methylene blue under high pressures. Even the cleavage lines previously mentioned were found to be impervious to the molecules of methylene blue when not subjected to pressure. Sections intentionally made to demonstrate these defects were used in the MCG and were found to

exhibit a brownish caries-like appearance, from the periphery of the enamel to the dentino-enamel junction within 3 to 5 days. It was believed at first that the appearance was merely a two-dimensional artifact. That it is in actuality a three dimensional verity is revealed in 3 sections which were made to include 2 cleavage lines which were less than 1 mm. apart. Within less than a week the sectors of enamel bounded by the 2 cleavage lines and the dentino-enamel junction fell away from the section. This was demonstrated on sections which were previously proved impervious to methylene blue stain after 3 weeks of immersion. The brownish structural change occurred only in those cleavage lines directly below the bacterial plaque on the surface of the tooth. This lends evidence to the fact that whereas the size of the molecules of methylene blue do not penetrate these microscopic clefts, the acids of bacterial metabolism are capable of ingress. If these enamel infractures are of significance to the rapidity with which certain lesions occur only further experimentation will reveal. Nevertheless, if cleavage lines were a decisive element in the caries complex we should expect to find lesions of a linear type corresponding to the directions of these lines, i.e., cervico-occlusally. Clinically, the author has observed cleavage lines to frequently bisect cavities, and rarely extend beyond the morsel curvature of the crown. However, it is important not to lose sight of the fact that cleavage lines may occur on all axial surfaces and are frequently quite evident on so-called susceptible areas where not the slightest evidence of a lesion is seen to exist. Hence, the importance of the facility with which cleavage lines appear to be affected, in the course of experimentation, remains questionable.

SUMMARY

1. An apparatus is described which is designed to permit the constant microscopic observation of plaque formation and pre-plaque phenomena as well as cavitation. The device is not subject to the usual limitations of *in vitro* methods because of the apparent nature of the carious lesion. However, it is not conceived to be an infallible automaton as certain features of artificiality cannot be denied.

2. A caries-like lesion can be produced in enamel by the method described if the saliva is adjusted to an average pH range of 6.0 and if a hydrolyzable carbohydrate substrate be added to ensure the local proliferation of bacteria of salivary origin.

3. The lesion is, at least in part, a product of the acidogenic action of the bacteria as evidenced by the fact that only the enamel immediately beneath the plaque is affected.

4. Saliva adjusted to pH 6.0 was not found to be of decalcifying potency for enamel, but is slightly so for dentin.

5. The zoöglea of bacteria constituting the experimental plaque is far more adherent to the enamel in saliva held within the lower pH ranges than the higher.

6. The growth of the plaque was observed to occur, as is expected, in a centripetal fashion when viewed in the apparatus designed for the observation of the carious lesion.

7. The polymicrobic character of the plaque, other than being demonstrated by direct smear and plating techniques, was directly observable by the method herein described.

8. The initially produced lesion is discrete and well localized beneath the most proliferated portion of the bacterial plaque. However, the lesion is much more caries-like in its incipiency than later when the acid attack instigated by the bacteria on the surface is more diffuse.

9. The leptothrix, yeasts, oidium, and perhaps other filamentous forms probably contribute nothing to the causation of the lesion for lesions were produced in their absence. Their late appearance on a lesion makes one inclined to believe that they are merely environmental opportunists.

10. Other than lactobacilli, numerous streptococci were consistently found in smears from the periphery of the enamel. However, the caries-like lesions produced are probably the result of the acidogenic participation of the total local flora.

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THE CHEMICAL COMPOSITION OF ENAMEL AND DENTIN IN HIGH AND LOW CARIES AREAS IN SOUTH AFRICA[1]

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INTRODUCTION

It is a recognised fact that dental caries is the most prevalent disease among civilized people. The experience rate is estimated to be over 95%. By this is meant that more than 95 out of every 100 persons suffer or have suffered at some time from one or more carious teeth.

The active etiological factor of dental caries is acid produced by the oral bacteria. There are, however, many contributory factors which influence the activities of these bacteria. Many research workers have explored various avenues to determine which of these contributory factors play the most important role in the problem of dental caries.

Armstrong and Brekhus (1937) and Bowes and Murray (1935, 1936) investigated the question of whether a variation in the chemical composition (except the fluorine content) of enamel and dentin affects dental caries, with negative results. As, however, the investigators mentioned above were not dealing with very pronounced variations in the incidence of dental caries, as well as in the chemical composition of drinking water supplies and soils, investigations on the subject were carried out in South Africa.

The incidence of dental caries (as defined above) among South African European school children varies from 10% in some areas to 100% in many other areas. The mineral content of the drinking water and soils in these areas also varies considerably.

This investigation was conducted on the following lines to determine: 1. Whether any variation exists in the calcium, magnesium, phosphorus and fluorine content of enamel and dentin in high- and low-caries areas in South Africa; and 2, whether the mineral content of the drinking water and soils in those areas affect the chemical composition of enamel and dentin.

MATERIALS AND METHODS

The high-caries area selected for this investigation was the Knysna district with a caries-experience rate of 99% and the low-caries area the Calvinia district, with a caries-experience rate of 40% (*fig. 1*).

APPEARANCE AND STRUCTURE OF THE TEETH

The teeth of the children in the Knysna district have a dull white, chalky appearance. The cusps are high and sharp, the grooves and fissures are deep

[1] Received for publication September 28, 1943.

[2] Proof read by Editor.

and often show a defective enamel structure. Food particles easily lodge in these deep grooves and fissures, making the teeth more prone to decay. Dental caries is rampant and there are many interproximal cavities in the lower incisors. The majority of the people become edentulous before they reach the age of 15 years. Of the 673 children examined, only 6 never suffered from caries. No cases of mottled enamel were seen.

The teeth and jaws of the children in the Calvinia district are well developed and strong in appearance. The teeth are lustrous and of a light cream colour. The cusps are low and flat. The grooves and fissures are shallow and exhibit no defective enamel structure, and this prevents food particles from lodging in them. Of 556 children examined, 335 never suffered from caries. One hundred and forty-five children had mottled enamel, varying in degree from very mild to moderate.

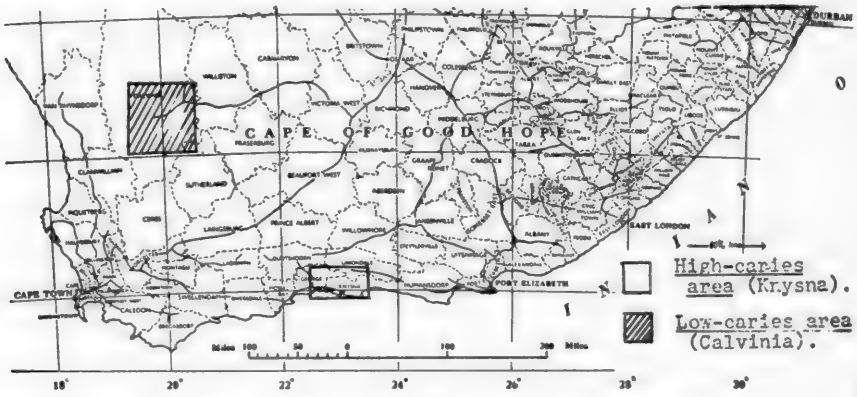

FIG. 1. Location of Knysa and Calvinia Areas in South Africa

THE DIETS AND THE AREAS

The diet of the people in the Knysna district consists mainly of *bread, rice, sweet-potatoes and coffee*, while that of the people in the Calvinia district consists mainly of *meat, bread and coffee with milk*. Table I gives a short description of the Knysna and Calvinia districts.

SOLAR RADIATION

No figures of the ultraviolet refraction are available for the Knysna and Calvinia areas, but Riemerschmidt (1940) reports that the hours of sunshine and the degree of daily solar intensity at the inland stations exceeded those at the coastal stations practically all the year round. From this may be deduced that the ultraviolet refraction figure for Knysna (coastal area) will be lower than for Calvinia (inland area).

TEETH ANALYSIS

Teeth extracted by local dentists and medical practitioners were collected and prepared for chemical analysis to determine the percentage CaO, MgO,

TABLE I

	KNYSNA	CALVINIA
Latitude	Between 33° 35′ and 34.5′ South.	Between 31° and 32° South.
Longitude	Between 22° 35′ and 23° 30′ East.	Between 19° 30′ and 20° 30′ East.
Altitude	Between 100–1,000 feet above sea level.	Between 3,000–4,000 feet above sea level.
Topography	Undulating and hilly. Cape coastal belt.	Flat. Bushmanland and upper Karroo plateau regions.
Geology	Table Mountain Series of the Cape System (quartzitic sandstones and shales).	Dwyka, Ecca and Beaufort Series of the Karroo System (tillite, sandstones and shales).
Climatology	Warm, temperate, winter and summer rainfall.	Hot, summer rainfall.
Hours of sunshine	Approximately 7.30 per day.	Approximately 9.73 per day.
Mean maximum temperature	70°F	76°F
Mean minimum temperature	56°F	46°F
Mean annual rainfall	28.38 inches.	7 inches.
Vegetation	Temperate evergreen forest.	Desert shrub, bush and succulents.
Soils and soil analysis:	Grey brown to light brown, sandy loam.	Light brown, gritty and pebbly clay loam, fairly loose.
SiO_2	85%	72%
CaO	0.15%	1.7%
MgO	nil—0.37%	2.8%
Na_2O	nil	1.6%
K_2O	nil	1.7%
P_2O_5	0.02%	0.07%
pH	4.9	8.2
Water supply and analysis:	Springs and rain water:	Mostly from boreholes:
pH	6.1	7.5
Total solids at 95°C	181 p.p.m.	1934 p.p.m.
Cl	181 p.p.m.	1934 p.p.m.
SO_4	48 "	397 "
CO_3	10 "	548 "
NO_3	9 "	142 "
F	nil	nil
Ca	0.19 "	1.5 "
Mg	5 "	121
Na	4 "	66
K	24	430
Total hardness as $CaCO_3$	1.4	1.5
	27	594
Farming activities	Timber growing.	Sheep and wheat farming.

TABLE II

DISTRICT	CARIOUS ENAMEL				SOUND ENAMEL			
	CaO	MgO	P_2O_5	F	CaO	MgO	P_2O_5	F
	%	%	%	*p.p.m.*	%	%	%	*p.p.m.*
Knysna	50.61	0.71	40.6	153	50.96	0.87	—	153
Calvinia	—	—	–	—	49.76	0.71	39.9	445
	CARIOUS DENTIN				SOUND DENTIN			
Knysna	38.44	1.51	30.67	252	39.34	1.61	29.80	268
Calvinia	—	—	—	—	38.26	1.60	29.86	755

TABLE III

Details of 40 samples of enamel and dentin analysed

CARIOUS ENAMEL—KNYSNA AREA					CARIOUS DENTIN—KNYSNA AREA				
No.	F	CaO	MgO	P_2O_5	No.	F	CaO	MgO	P_2O_5
	p.p.m.	%	%	%		*p.p.m.*	%	%	%
1	200	51.86	0.79	—	19	150	38.13	1.73	—
2	180	52.15	0.76	—	20	340	37.85	2.14	—
3	60	50.12	0.64	—	21	370	39.87	1.72	—
4	100	49.94	0.83	—	22	100	37.73	0.86	—
5	70	51.30	0.68	41.1	23	250	37.75	1.57	—
6	160	51.50	0.58	41.0	24	140	37.80	1.52	29.6
7	230	49.50	0.68	40.8	25	200	38.90	1.61	31.2
8	70	50.30	0.66	41.2	26	520	39.40	1.41	31.9
9	310	48.80	0.76	39.0	27	200	38.50	1.28	30.0
Aver.	153	50.61	0.71	40.6	Aver.	252	38.44	1.51	30.67
SOUND ENAMEL—KNYSNA AREA					SOUND DENTIN—KNYSNA AREA				
10	180	53.33	0.64	—	28	300	39.25	1.71	—
11	150	49.09	0.86	—	29	320	39.95	2.08	—
12	130	50.46	1.12	—	30	200	37.62	1.30	—
					31	320	42.10	1.50	—
					32	200	37.20	1.48	29.80
Aver.	153	50.96	0.87	—	Aver.	268	39.24	1.61	29.80
SOUND ENAMEL—CALVINIA AREA					SOUND DENTIN—CALVINIA AREA				
13	750*	49.27	0.77	—	33	1700*	39.35	1.30	—
14	250	51.20	0.72	40.1	34	500	37.50	2.20	30.1
15	700	48.90	0.66	40.5	35	500	38.00	2.16	28.3
16	620	48.50	0.73	39.0	36	900	37.90	1.67	31.2
17	150	50.00	0.56	39.9	37	980	37.30	1.88	28.9
18	200	50.70	0.81	39.9	38	980	39.70	1.37	31.7
					39	350	38.00	1.22	28.8
					40	130	38.30	1.00	30.0
Aver.	445	49.76	0.71	39.9	Aver.	755	38.26	1.60	29.86

* Mottled enamel and dentin.

P_2O_5 and F. The decayed material, when present, was first removed with burs. The crowns were then broken into small bits, the pulps removed and the enamel and dentin separated by the flotation method as described by Manly and Hodge (1939). These analyses were done by the Division of Chemical Services, Department of Agriculture and Forestry, Pretoria.

Table II gives the average results of the chemical analysis of samples of carious and non-carious enamel and dentin of teeth from people living in the Knysna and Calvinia areas. The details are given in Table III.

DISCUSSION

The results of the chemical analysis of the enamel and dentin from the high- and low-caries areas show no significant difference in the percentage CaO, MgO and P_2O_5. These results correspond to the findings of Armstrong and Brekhus (1937) and Bowes and Murray (1935, 1936) as Table IV indicates.

TABLE IV

INVESTIGATOR	CARIOUS ENAMEL			SOUND ENAMEL		
	Ca	Mg	P	Ca	Mg	F
Armstrong & Brekhus	35.64	0.32	17.21	35.41	0.30	17.45
Bowes & Murray	—	—	—	37.07	0.464	17.22
Division of Chemical Services	36.15	0.426	17.72	35.54	0.426	17.42
	CARIOUS DENTIN			SOUND DENTIN		
Armstrong & Brekhus	—	—	—	26.18	0.83	12.74
Bowes & Murray	27.98	1.26	13.87	27.79	0.835	13.81
Division of Chemical Services	27.45	0.90	13.39	27.33	0.96	13.03

Although the calcium, magnesium and phosphorus content of the drinking water and soils of the Knysna area are very low and in the Calvinia area very much higher, they do not appear to affect the calcium, magnesium and phosphorus content of the enamel and dentin of people living in those areas.

The fluorine content, however, is considerably higher in the enamel and dentin in the low-caries area than in the high-caries area. It probably has its origin in the drinking water (1.5 p.p.m.). The amount of fluorine in the drinking water affects the fluorine content of the enamel and dentin (more so the dentin) and also influences the caries-incidence rate. The effect of fluorine in the drinking water on dental caries has been discussed by many investigators amongst whom may be mentioned Volker and Bibby (1941), Irving (1941) and Wilson (1941).

Dean, Jay, Arnold, Elvolve and McClure (1939, 1941), Silva, Chapedi and Pedace (1940), Wilson (1941) and the author (1941, 1942) have reported a lower incidence of dental caries in the endemic fluorine areas than in the non-endemic areas.

It appears that an optimum amount of fluorine in the drinking water assists the proper calcification of the teeth making them more resistant to caries. Whether the diet, fluorine, hardness of the drinking water and the degree of

solar intensity (including vitamin D) also play a part in a synergistic action on the caries-incidence rate merits further investigation. It may be of interest to mention that the skulls of Bushmen found in caves along the coastal areas of Knysna show no evidence of dental caries. These Bushmen were hunters as evidenced by the bones of animals (mostly buck) found in the middens. Their diet consisted chiefly of meat. They drank water from the same sources as the people living in those areas to-day.

SUMMARY

Forty samples of carious and non-carious enamel and dentin from people in high- and low-caries areas in South Africa were analysed for calcium, magnesium, phosphorus and fluorine. No significant difference was found in the percentage of calcium, magnesium and phosphorus.

The fluorine content, however, was considerably higher in both the enamel and dentin of people in the low-caries area (445 p.p.m. and 755 p.p.m. respectively) than of people in the high-caries area (153 p.p.m. and 258 p.p.m. respectively). This higher fluorine content of the enamel and dentin corresponds with the higher fluorine content in the drinking water. The calcium, magnesium and phosphorus content of drinking water and soils appears to have no effect on the calcium, magnesium and phosphorus content of enamel and dentin.

It is desired to thank Dr. Peter Allan, Secretary for Public Health, for permission to submit this article for publication. Thanks are due to the Chief of the Division of Chemical Services, Department of Agriculture and Forestry, Dr. J. P. van Zyl and his staff for the chemical analyses of the soils, water and teeth.

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THE ACTION OF SODIUM FLUORIDE ON THE DENTIN AND PREDENTIN OF THE INCISOR TEETH OF RATS CONSUMING DIETS CONTAINING CALCIUM AND PHOSPHORUS IN VARIOUS RATIOS[1]

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The effects of NaF upon normal teeth are by now well known, and the histological changes have been described by Schour and Smith (13, 14). The observations recorded in the present paper arose as a result of some investigations into the action of vitamin D upon the teeth of rachitic rats (Irving, 7). In these experiments, animals subsisting upon diets of normal, high or low Ca:P ratio were given vitamin D by mouth, and some were injected with 2% NaF solution at the same time, to act as a marker on the tooth. In order to interpret the result of 1 injection of NaF, control animals, receiving the diets alone, were examined and showed results, some of which did not accord with current thought and some of which had not been before described.

The present experiments were therefore undertaken to investigate in more detail the early action of NaF upon the dentin, using the same diets as those previously employed. A preliminary report of some of these findings has already been published (Irving, 6).

EXPERIMENTAL

Diets. Three diets were used. Diet 1 was the stock diet given to the rat colony in this department. It was made up of mixed cereals, bone and fish meal, oat germ meal and cod liver oil and was a complete dietary. Diet 2 was the Steenbock and Black rachitogenic diet (15). Diet 3 was prepared by modifying the Steenbock and Black diet by reducing the $CaCo_3$ and adding Na_2HPO_4 so that the Ca:P ratio was approximately 0.25. The following was the Ca and P content of the diets, the figures being checked by analysis.

Diet no.	*Ca%*	*P%*	*Ca: P*
1	1.23	0.84	1.5
2	1.21	0.28	4.4
3	0.122	0.42	0.29

Diet 1 was prepared in cubes and fed as such. Diets 2 and 3 were given as a powder slightly moistened with water.

Rats. The animals used were Wistar Institute strain albino rats, kept in a

[1] The expenses of this work were defrayed by grants from the Staff Research Fund, University of Cape Town, and the National Research Council and Board, Union of South Africa. Received for publication October 27, 1943.

[2] At the request of the author proof was read by Dr. Isaac Schour, University of Illinois, Chicago, Ill.

special animal house at a temperature of 68°–70°. Males and females were used indiscriminately, the results found being the same in both sexes. After weaning at 25 days, the young rats were housed by litters in steel cages and fed on the standard stock diet used in the department (diet 1). In the case of diets 2 and 3, the young rats were transferred to these diets when between 50 and 60 g. in weight, and maintained on them for 28 days. They were then injected once with NaF solution. Of the rats on diet 1, some were injected when they were the same weight as the animals were after 28 days on diets 2 and 3, and others were injected when they were the same age as these animals at this stage. Three litters of rats on diet 1 were injected, 3 litters on diet 2 and 4 litters on diet 3.

Sodium fluoride injections. All animals were injected subcutaneously with 2% NaF solution. Those on diet 1 were given 0.2 to 0.3 c.c., depending on their weight. Those on diet 2 were given 0.25 to 0.28 c.c. This dosage was fatal in a few cases, the animals going into a comatose condition before death, but with no signs of tetany. Those on diet 3, which were usually heavier than those on diet 2, had to be given a greatly reduced dose as they almost all died in tetany if given the same amount of NaF as the other rats. The dosage finally employed was 0.05 to 0.1 c.c., and even with this dose convulsive seizures, not necessarily fatal, often occurred.

Time-Table used. The animals were killed at 6 hourly intervals up to 72 hours after injection. The litters used consisted initially of 6 or 8 rats, but some died prematurely as a result of the injection. The final scheme adopted is shown in this table:

DIET NO.	TIME KILLED AFTER INJECTION (Hours)							
	6	12	18	24	30	36	48	72
1	A	A	A	A	A	A	—	—
	B	B	B	B	B	B	B	B
	C	C	C	C	C	C	—	—
2	D	D	D	D	D	D	D	—
	—	E	E	E	—	—	—	—
	F	F	F	F	F	F	—	—
3	GG	G	G	G	G	G	—	—
	—	H	H	—	—	—	—	—
	I	I	I	I	I	I	I	I
	J	J	J	J	J	J	J	J

Histological Examination. After death, the skulls were fixed in formol-saline and decalcified with 2.5% nitric acid in formol-saline. The upper incisor teeth were cut in longitudinal section at 12 μ and stained with hematoxylin and eosin. The changes in the labial dentin and predentin were measured with a micrometer eye-piece, the changes on the lingual side being noted qualitatively. The

interpretation of hematoxylin-staining areas in teeth as well calcified, and of areas staining poorly with this stain, but well with eosin, as hypocalcified, has been shown to be valid by Schour and Ham (12) and by Schour, Chandler and Tweedy (10).

RESULTS

The animals on diet 1 were in all respects normal healthy rats. The general effects of diets 2 and 3 on rats have been already described (Irving,7) and are not germane to the subject of this paper. In both groups, the growth rate was very slow, being somewhat better with diet 3. All the rats on diet 2 had severe rickets; those on diet 3, a reduction in the bone ash, but no gross epiphyseal changes.

The histological changes in the incisor teeth of the rats of the various groups were identical in each litter. The following description summarises the main findings.

Diet 1.

Enamel. In all animals, no matter how early killed, the same changes were found in the enamel organ and the organic enamel as have been already described by Schour and Smith (13).

Distal dentin. The same changes were seen at the same times in both labial and lingual dentin and predentin. 6 and 12 hours: No change was seen in either dentin or predentin. 18 hours: A purple-stained hypercalcified line was found in some parts of the more distal predentin in the late predentin zone (*fig. 1*), 24 hours: A purple-stained line was present in the predentin in the distal part of the tooth. It was wider and further from the odontoblasts. A hypocalcified stripe was present between this line and the old dentin and was apparently the same as the predentin occupying this position 18 hours after injection. 30 to 72 hours: The hypercalcified stripe became wider, but was preceded throughout by the hypocalcified stripe, which appeared to be equivalent to the predentin already mentioned (*fig. 2*). By 72 hours, the hypercalcified parts of the stripe had merged with the now normally calcifying dentin, but was often separated from it by a narrow zone staining a characteristic sky-blue colour with hematoxylin. After 30 to 36 hours, the predentin width was again normal.

The changes seen are shown diagramatically in *fig. 7*. The zone *bc* was first found as a narrow line 18 hours after injection, when *ad* represented the total predentin width. In succeeding sections *cd* remained fairly constant in width, but *bc* became wider with incremental calcification, *ab* becoming predentin of normal width at and after 30 hours. A fine hypercalcified line was seen at *d*, the edge of the old predentin, in many sections at and after 18 hours. This has been called the calciotraumatic line by Schour, Chandler and Tweedy (10).

In the graph in *fig. 8* the distances *ac* and *ad* on the labial side are plotted in μ against time. The values for *ac* fall very nearly on to a straight line, which, when extrapolated, runs through the origin. This shows that the point *c* in the hypercalcified stripe corresponded in time with the injection. It will be noted that the distance *ac* increases at the rate of about 15 μ per 24 hours. The values

for *ad* are more irregular but tend to form a line paralled with that of *ac*, which, when extended, strikes the abscissa about 16 hours before the origin. This finding indicates that the predentin already formed up to about 16 hours before the injection, calcified but slightly, this stripe being bounded externally by the calciotraumatic line.

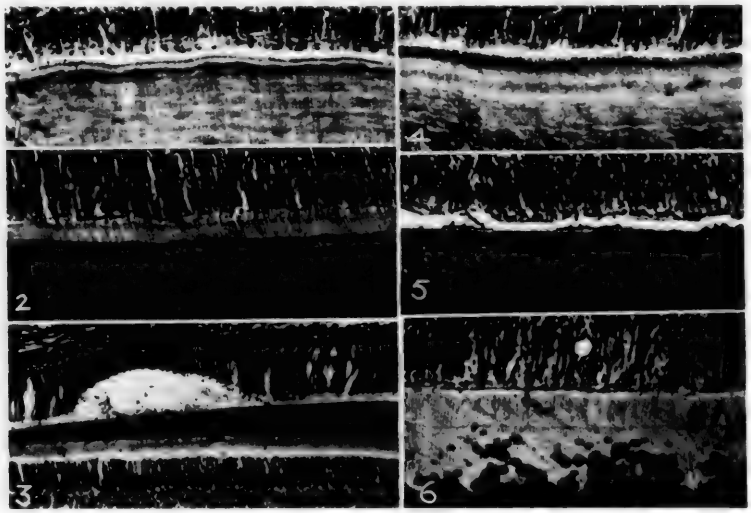

FIG. 1. Distal lingual dentin and predentin (diet 1). Animal had 1 injection of NaF 18 hours previously. Note hypercalcified line in predentin and calciotraumatic line.

FIG. 2. Intermediate labial dentin and predentin (diet 1). One injection of NaF was given 36 hours previously. This section shows calciotraumatic line, and hypo- and hypercalcified stripes.

FIG. 3. Extreme proximal labial region (diet 1). Animal had been injected with NaF 36 hours previously. Fine calcified line is seen in part of predentin. Distortion of ameloblasts is artefact.

FIG. 4. Distal labial dentin and predentin (diet 2). Injection of NaF was given 48 hours previously and reactive hypercalcified stripe is seen in dentin.

FIG. 5. Proximal labial dentin and predentin (diet 3). NaF was injected 18 hours before death. Arrow points to narrow calcified line in predentin.

FIG. 6. Intermediate labial dentin and predentin (diet 3). Animal had been injected with NaF 10 days previously (Irving, 7). Arrow points to calcified line which is now much farther from odontoblasts than in *fig. 5*.

All figures show longitudinal sections of part of the upper incisors, and the original magnification throughout was × 120.

Proximal dentin. In all sections at and after 18 hours, a very fine hematoxylin staining line was found for a short distance in the extreme proximal predentin on the labial side (*fig. 3*). This line moved slowly with time away from the odontoblasts and towards the calcified dentin and had become incorporated into it by 72 hours. The rate of movement was approximately 10 μ per 24 hours. The values for the distance of this line from the odontoblasts also formed a

straight line when plotted against time and intersected the origin when extrapolated, this showing likewise that the fine line was formed at the time of NaF injection. This line was not readily seen in the lingual predentin.

Diet 2. The same changes were seen in the enamel organ and in the organic enamel as occurred with diet 1. No changes of any kind were seen in the predentin, which was very wide proximally but somewhat narrower distally. The

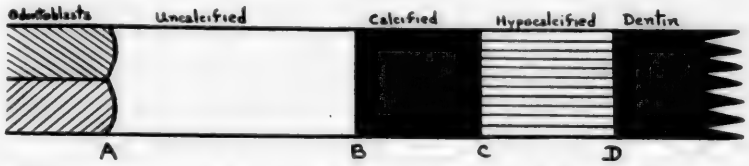

FIG. 7. Diagram of action of fluorine on predentin of animals on diets 1 and 3.

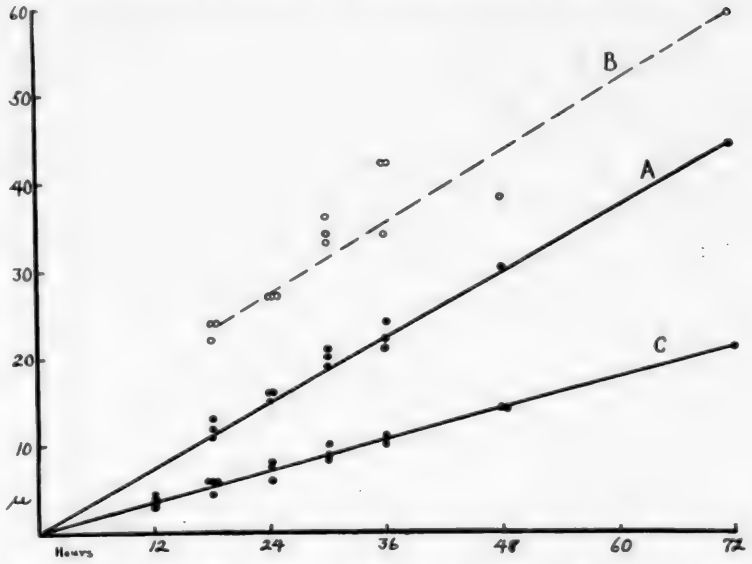

FIG. 8. Diagram showing time relations of changes in predentin of animals on diets 1 and 3. A: *ac* of animals on diet 1; B: *ad* of animals on diet 1; C: *ac* of animals on diet 3.

distal dentin showed a hypercalcified stripe 30 to 48 hours after the injection (*fig. 4*); this was sometimes preceded by a hypocalcified stripe.

Diet 3.

Enamel. The same changes were seen in the enamel organ and the organic enamel as occurred with diet 1.

Dentin. 6 hours: No changes seen. 12 hours: A fine hematoxylin staining line was seen in the proximal labial predentin only, in the early predentin zone. 18 and later hours: The fine line moved slowly outwards with the incremental growth of the predentin, passing out of the early predentin area into the late

predentin (*fig.5*). By 24 hours a similar line was present in the lingual predentin. Also, with the passage of time, the line extended further distally until by 72 hours it was found in the proximal half of the predentin on both sides. In later sections the distance of the calcified line from the odontoblasts was greater in the extreme proximal region, than in the intermediate parts of the tooth, indicating that the incremental rate was greater in the proximal part; in the intermediate part the distance was extremely constant in all places. The calcified line was still present 10 days after the injection (*fig. 6*) and in some cases would apparently have taken up to 20 days before being incorporated into the calcified dentin (Irving, 7). No change of any kind was seen in the dentin already formed.

In most sections the line appeared as a homogeneous structure, but in a few it had a cross-hatched appearance rather like a paling fence, indicating some disorganization in the organic matrix at this point. The predentin was quite uncalcified as judged by the staining appearance on either side of the line. The same type of pattern was seen as depicted in *fig. 7*, save that *bc* was always narrow and the distance *ad* so great that *bc* took up to 20 days to traverse it. The distance *ac* on the labial side is plotted against time in *fig. 8*. It will be seen that the points fall on to a straight line and that the incremental rate is about half that of normal teeth. The line joining the points runs through the origin when extrapolated, so that, as with diet 1, the calcified line was laid down at the time of the injection.

DISCUSSION

The changes in the enamel organ and organic enamel, using all 3 diets, were identical and already conspicuous 6 hours after the injection of NaF, when the dentin was still unaffected. This confirms the findings of Schour and Smith (13) that the ameloblasts are the first cells to be affected by this substance; and the fact that the teeth of the animals on diet 2 showed changes in the organic enamel but none in the predentin at any time also shows that the ameloblasts are much more sensitive to NaF than the odontoblasts.

The changes in the distal predentin and dentin in the teeth of animals on diet 1 are not difficult to analyse. The injection of NaF affected in some way the predentin forming at the time so that it began to calcify after 18 hours and not after 24 hours, the first dentin forming in this way being hypercalcified. Rather more than half of the predentin already existing at the time of the injection apparently calcified only slightly, if at all, and persisted as a hypocalcified stripe and was often bordered by the calciotraumatic line. Such cessation of calcification also occurs in other conditions where some Ca metabolic change takes place. It is thus sometimes seen at the neonatal line (Kronfeld and Schour, 8, fig. 6), at the time of parathyroidectomy (Schour, Chandler and Tweedy, 10) and also when Mg is added to a Mg deficient diet (Irving, 5).

The measurements quoted above show that the outer edge of the hypercalcified stripe represents predentin forming at the moment of injection of the NaF. This is particularly confirmed by the fact that the extrapolation of the line in *fig. 2* cuts through the intersection of the ordinate and abscissa. The calcio-

traumatic line and hypocalcified stripe, while caused by the NaF, are formed in predentin laid down up to 16 hours before the injection. Thus the statement of Schour and Smith (13) that the hypocalcified layer represents an immediate response to the injection and the dark layer a recovery response is not completely confirmed, only the later parts of the hypercalcified stripe being formed during recovery from the injection. The calciotraumatic line has been described by Schour, Chandler and Tweedy (10) as an expression of a shock to Ca metabolism; it is not, however, any more of this nature than the other changes in the predentin and dentin. It appears much simpler to regard the entire pattern of calciotraumatic line, hypo- and hypercalcified layers as but one response to NaF.

The calcified line that was found in the proximal predentin of animals on diet 1 corresponded in time to the injection. The response in this part of the tooth, which has not been described before, appeared at the same time as that in the distal predentin, and must presumably be produced by a similar mechanism. The calcified line in the predentin of animals on diet 3 would appear likewise on the basis of the measurements quoted above, to have been formed at the time of the injection of NaF. In the case of the animals on diet 1, whose distal predentin was soon calcifying, no break was seen between the hypercalcified line and the new dentin, but with the animals on diet 3, calcification only occurred while the NaF was acting, and, judging from the width of the line, this can have been for only a few hours; after the NaF action had ceased, the predentin relapsed into the non-calcified state already imposed on it by the diet. The same presumably applies to the proximal predentin of the animals on diet 1, and the extreme narrowness of the line suggests that here too the fluorine had acted for only a short time.

The predentin of the animals on diet 2 showed no calcified line, but must presumably have been affected by the NaF since a hypercalcified line occurred in the dentin formed from this predentin when it ultimately calcified. If the animals were allowed to live for 10 days after the injection, a well-marked NaF line was found in the distal and intermediate dentin (Irving, 7); this was often preceded by a hypocalcified layer, which suggests that the pattern of fluorine action was the same as with diet 1.

It is of interest to note that the part most sensitive to NaF of the teeth of animals on diet 3 was the proximal labial predentin. The proximal lingual predentin was the next in order of sensitivity and later the more distal parts of the teeth were affected. In animals on normal ratio diets, on the other hand, the distal parts of the tooth were early sensitive to NaF, as has been pointed out by Schour and Smith (14); but the extreme proximal predentin appeared to be almost as soon affected in these rats as in those on diet 3. It is also of interest to note that the dentin apposition rate in animals on diet 1 was faster distally than proximally, whereas in animals on diet 3, the reverse held. The predentin of the animals on diet 3 showed a very clear demarcation into early and late predentin. The NaF line was present in the early predentin in animals killed at 12 hours, showing that calcification could also occur in this zone.

Two further considerations arise from the above findings: the first is the

relationship between the diet and the appearance or non-appearance of the calcified line in the predentin; the second is the relevance of these findings to dentin calcification in general.

It seems quite clear that the Ca/P ratio of the diet and thus the level of the blood Ca was the controlling factor in the appearance of the calcified line in the predentin. Thus, in the animals on diet 3, whose liability to tetany showed that their blood Ca must have been very low, the line appeared after 12 hours. In the animals on diet 1, consuming a diet of normal ratio, the line appeared after 18 hours, when a dose of more than twice the amount of NaF was used. While the teeth of the animals on diet 2 appeared the most insensitive to NaF, these animals presumably had the highest blood Ca as they showed no signs of tetany even when the amount of NaF given was fatal. The protective action of Ca against F intoxication has been already remarked by Lawrenz and Mitchell (9). The predentin of animals on diet 3 has been shown to differ also in its reaction to vitamin D from that formed while the animals are on diets 1 or 2 (Irving, 7).

The action of NaF on normal animals must be associated with a lowering of the blood Ca and it would appear from the above data that a condition in which the blood Ca is already lower than normal predisposes to a more forceful and earlier action of fluoride. It is at present impossible to correlate this finding with any of the known theories of calcification. In fact most of these associate calcification with a local elevation of the Ca ions. Schour and Ham (11) found that the hypercalcified stripe after dosage with vitamin D or parathormone was formed when the blood Ca was falling after an initial rise, but this is not quite parallel to the conditions existing here. Schour and Smith (13, 14) considered that NaF acted primarily on the odontoblasts, but it would seem from what has just been said, that a large part of its action on the tooth must be through changes in the blood composition.

It does not seem likely that the hypercalcified area is a deposition of calcium fluoride. Judging by the behaviour of the animals, and the width of the line in the proximal predentin of the animals on diets 1 and 3, the toxic action of the fluoride was over at latest 6 hours after the injection and yet the hypercalcified line was not seen till 18 hours after the injection with diet 1 and 12 hours with diet 3. It had thus been present in a form not staining with hematoxylin for several hours. In the case of the animals on diet 2 this invisible condition persisted till calcification occurred. It would thus seem more probable that the predentin had been affected in some way which altered normal calcification.

Arising from these considerations, is the second query mentioned above, namely, how is dentin normally calcified and what governs this? The calcification of enamel has recently been studied in some detail (1, 2, 3, 4, 16, 17) and the almost universal conclusion reached is that this change does not occur till the organic enamel matrix is fully formed. Diamond and Weinmann (4) suggest that calcification may be by the crystallisation of Ca salts already present in colloidal form, though if this is so the entire matrix containing such salts should be in some degree radiopaque. Schour and Ham (12) have produced

good evidence that predentin is not calcified and it seems to be generally accepted that the dentin becomes more calcified as the odontoblasts recede, the mantle dentin being the most calcified. It is not possible to picture with our present knowledge how the elements of calcification reach the calcifying dentin; but one can speak with more assurance of the factors controlling this calcification. Diamond and Weinmann (4) consider that the agents affecting calcification of enamel, such as those causing hypoplasia, act on the matrix as it is forming and not as it is calcifying. The same appears to hold with regard to dentin. Fluorine cannot act at all on dentin actually calcifying when the injection is given. This is seen in all the 3 diets used and especially with diets 2 and 3. In the case of normal animals, predentin laid down up to 16 hours before F administration can be affected, as is seen by the calciotraumatic line and the zone of hypocalcification. But if the predentin has existed for more than 16 hours then it is outside the pale of the influence of F and probably also outside the influence of most agents affecting calcification. Some property has been laid down in the predentin during formation which determines its calcification at a certain time: in 24 hours under normal circumstances, and later or earlier in various disturbances of Ca metabolism.

In conclusion, it appears that the wide predentin of animals on such diets as no. 3 is a very useful background on which to study calcification phenomena. It is more sensitive than normal predentin and remains uncalcified for a considerable time. It is hoped to pursue studies of calcification further using this technique.

SUMMARY

1. This paper records the studies of the early action of fluorine on the incisor teeth of rats consuming diets of normal, high and low Ca:P ratio (diets 1, 2 and 3 respectively).

2. A hypercalcified line was first seen 18 hours after NaF injection in the distal predentin of the incisors of rats on diet 1. Measurements showed that the first part of this stripe was formed in predentin which was being laid down at the time of the injection. The stripe moved outwards with the incremental growth of the dentin and was preceded by a hypocalcified layer and the calciotraumatic line of Schour, Chandler and Tweedy. These latter 2 zones were formed in predentin laid down up to 16 hours before the NaF injection. In addition, a fine calcified line was found at and after 18 hours in the extreme proximal predentin. This line was also formed at the time of the injection.

3. The wide predentin of animals on diet 2 showed no calcification changes after NaF injection. The dentin had a hypercalcified stripe when the animals were allowed to live long enough for the matrix forming at the time of the injection to become calcified.

4. The proximal labial predentin of animals on diet 3 showed a fine calcified line 12 hours after the injection of NaF. This moved outwards with the incremental growth of the predentin and later a similar line was seen in the proximal lingual predentin and finally in the intermediate predentin on both sides. Meas-

urements showed this line to be present in predentin forming at the time of the injection. The animals on this diet were very liable to tetany after NaF injection.

5. The above results are discussed with relation to the action of fluorine on the teeth and to some problems of dentin calcification. It is concluded that fluorine acts primarily by altering the composition of the blood and that the calcification changes produced thereby are variants of the normal process.

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EFFECTS OF LARGE DAILY DOSES OF VITAMIN D ON TEETH AND JAWS OF RATS AND ON HUMANS[1]

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The purpose of this study was to determine the effect of massive daily doses of vitamin D[2] on dentin apposition, bone apposition, tooth eruption, calcification rhythm, skeletal calcification, pulp stone formation, estrus cycle, and toxicity in rats, and pulp stone formation in humans. [Experiments in the treatment of rheumatoid arthritis with massive daily doses of vitamin D have been conducted by a number of investigators (1).]

Schour and Ham (2) reported the effect on the calcification of the incisor dentin of the rat from the administration of a single large dose of vitamin D. Schour and Hoffman (3), in a study of dentin calcification rhythm, reported that severe experimental interferences such as hypophysectomy or parathyroidectomy did not alter the calcification rhythm. Weinmann (4) studied calcification and pulp stone formation by histological section. The methods employed in the present study differed from those used in the aforementioned. To the best of our knowledge, the literature does not report a comparable experiment.

ANIMAL EXPERIMENTS

Thirty-two female, white rats of the Sherman strain, aged 57 to 59 days at the beginning of the experiment, were observed. Twelve served as an untreated group, while 20 were treated experimentally. There were 4 sets of litter-mates, 4 animals to each set, distributed between the untreated and treated groups in equal numbers. The study was set up so as to provide 2 sets of controls; namely, the untreated group of animals, and a control period in each animal. A control period of 2 weeks was followed by an experimental period of 2 weeks. In this way, a safe margin was allowed for the time required for the incisor of the rat to renew itself. Only 4 animals were placed in a cage, thus giving each a better opportunity to obtain ample food. The animals were earmarked and weighed, and each cage was provided with a regular stock diet.[3]

[1] Presented at a meeting of the Jarvie Society, New York, May 1st, 1942.

Received for publication, July 26, 1943.

[2] Ertron, a vitamin D preparation of exceptionally high potency, prepared by the Whittier process (activation of heat-vaporized ergosterol by electrical energy). The materials were furnished and the investigation was partially supported by a grant from the Nutrition Research Laboratories, Chicago, Ill.

[3] Alfalfa leaf meal, 2%; whole milk powder, 10%; ground whole wheat, 15%; pearled barley, 15%; shelled oats, 15%; yellow corn, 15%; soya bean meal, 15%; meat scraps, 10%; sodium chloride, 2%; calcium carbonate, 0.5%.

The rats were weighed at the beginning of the experiment, at the end of the control period (first 2 weeks), and again at the end of the experimental period (second 2 weeks).

Because of the favorable reports of Reed and others (1) on the comparative safety of the Whittier-process type of vitamin D and because it was being tried on patients in the Arthritis Clinic at the Medical Center, Columbia University, this product (Ertron) was chosen as the experimental agent. Each rat of the twenty received daily 10,000 U.S.P. units incorporated in the diet, the mixture fed being weighed and consumption time recorded. There was some loss owing to unavoidable wastage so that the estimated amount ingested daily was about 7,000 U.S.P. units.

The weights of the rats (Table I) vary from 100 to 170 grams, the average starting weight being 128 grams (130 for untreated, 126 for treated animals). The daily dose per kilogram of body weight was between 60,000 to 100,000 U.S.P. units. For the rat of average weight (128 grams) the daily dose per kilogram of body weight was about 78,000 U.S.P. units. No specific attempt was made to determine the minimal toxic dose. It is significant, however, that none of the animals showed grossly observable signs of toxicity or deleterious effects while receiving the vitamin D preparation.

Dentin Apposition Rate

The vital staining method (5) was used for measuring the amount of dentin apposition. Alizarin Red S was injected intraperitoneally, 100 mgm. for each kilogram of body weight, at the beginning of the control period. This was repeated at the beginning of the experimental period, and again at the end of the experimental period.

Ground histologic sections were made of each mandible to study the appositional growth of dentin by means of the alizarin lines. These sections were made by cutting 5 cross-sections from one-half of the mandible and 1 longitudinal-section from the other half in such a way that the incisor tooth and jaw bone were visible in each section. From each slide, sections were selected where 3 adjacent alizarin lines were present in the dentin, indicating the dentin apposition during the control and experimental periods. The distance between these lines was measured along the dentinal tubules, measuring between the pulpal sides of the alizarin lines with an eye-piece screw micrometer.

Results. There was an increase in the dentin apposition rate in both groups of rats during the experimental period. In the untreated animals the increase was slight but significant (0.94 micra per day), whereas the treated animals showed an accelerated dentin apposition rate (2.33 micra per day) (Tables II and VI).

There was no observable change in quality of dentin as observed microscopically with methods of preparation described above (*figs. 1, 2, 3, 4*).

Bone Apposition

The vital staining method, as described under *Dentin Apposition Rate*, was employed. Bone measurements were made perpendicular to the surface in sec-

TABLE I

Weight in grams

RAT	CAGE	(CONTROL PERIOD) (EXPERIMENTAL)		
		Start	Mid	End
Untreated animals				
Plain	2	110	150	145
NL	2	135	160	155
NR	2	130	150	170
SR	2	140	165	175
Plain	4	135	155	165
NL	4	140	150	170
NR	4	140	140	160
SR	4	125	155	170
Plain	8	130	150	175
NL	8	130	160	175
NR	8	125	150	185
SR	8	125	150	175
Totals		1565	1835	2020
Mean		130	153	168
Mean gain			22	15
σ_m			3	3
Critical ratio			8.0	4.7
Treated animals				
Plain	1	130	150	155
NL	1	130	150	160
NR	1	140	155	165
SR	1	130	155	130
Plain	3	140	150	170
NL	3	140	160	165
NR	3	145	150	150
SR	3	145	165	170
Plain	5	105	130	140
NL	5	100	120	135
NR	5	150	165	165
SR	5	120	135	135
Plain	6	110	130	135
NL	6	115	135	150
NR	6	140	155	170
SR	6	100	125	135
Plain	7	100	150	165
NL	7	110	140	155
NR	7	110	125	135
SR	7	170	195	200
Totals		2530	2940	3085
Mean		126	147	154

TABLE I—*Continued*

RAT	CAGE	(CONTROL PERIOD) (EXPERIMENTAL)		
		Start	Mid	End
Treated animals—*Continued*				
Mean gain		20	7	
σ_m		2	2	
Critical ratio		4.4	3.4	
*Treated-untreated		−2	−8	
σ_m		3	4	
Critical ratio		0.6 (not significant)	2.1 (not significant)	

* Note: While the *rate* of weight increase declines during experimental period as compared with control period, and the rate of decline is a little bit greater in treated animals, when the differences are compared between the treated and untreated animals, the result is insignificant.

tions where 3 adjacent alizarin lines were present, indicating the bone formation during the control and experimental periods.

Results. A marked *increase* in bone formation was observed in the treated animals during the experimental period as compared to the amount of apposition seen during the control period when no vitamin D was administered. No such increase occurred in the untreated rats. The bone formation in the 12 untreated animals showed a slight but significant *decrease* in amount during the experimental period as compared to that found during the control fortnight. Since bone is not formed at an equal rate in all areas, increases were determined in terms of percentages rather than in terms of absolute figures so as to compensate for these appositional differences. Statistical examination at this phase of the study revealed the percentage increase to be significant—34.4% (Tables III and VI).

There was no observable change in the quality of bone as observed microscopically (*figs. 5* and *6*).

Calcification Rhythm

To observe the calcification rhythm in the dentin, the perpendicular distance between successive contour lines of Owen was measured. Measurements were made of the dentin formed during the control period as well as that formed during the experimental period, determined by the presence of the alizarin lines.

Results. Calcification rhythm was found to vary both in the untreated and the treated animals from 10.0 to 19.8 micra, with the median at about 15 micra. The differences in calcification rate during the control and experimental periods in both groups of animals were computed (Table IV). While variations in measurements were noted in both groups, the measurements for each animal were comparatively constant. These results indicate that the experimental procedure had no significant influence on calcification rhythm (Table VI).

TABLE II

Dentin apposition rate

RAT	CAGE	CONTROL PERIOD	EXPERIMENTAL PERIOD	DIFFERENCE
Untreated animals				
		micra/day	*micra/day*	*micra/day*
Plain	2	14.2	16.7	2.5
NL	2	15.5	16.1	0.6
NR	2	16.4	16.4	0.0
SR	2	16.4	16.6	0.2
Plain	4	14.9	15.8	0.9
NL	4	15.2	16.3	1.1
NR	4	15.8	17.5	1.7
SR	4	12.5	14.6	2.1
Plain	8	15.8	15.8	0.0
NL	8	16.4	16.6	0.2
NR	8	14.2	15.7	1.5
SR	8	14.4	14.9	0.5
Totals		181.7	193.0	11.3
Mean		15.1	16.1	0.94
σ				0.81
σ_m				0.23
Treated animals				
Plain	1	14.5	16.5	2.0
NL	1	15.2	16.1	0.9
NR	1	14.1	17.2	3.1
SR	1	14.5	15.8	1.3
Plain	3	14.7	17.1	2.4
NL	3	14.1	14.5	0.4
NR	3	13.4	16.2	2.8
SR	3	12.7	16.4	3.7
Plain	5	14.5	17.7	3.2
NL	5	14.5	16.0	1.5
NR	5	13.6	16.8	3.2
SR	5	13.8	14.9	1.1
Plain	6	13.8	18.2	4.4
NL	6	14.5	17.5	3.0
NR	6	—	—	—
SR	6	15.8	18.0	2.2
Plain	7	14.4	16.0	1.6
NL	7	12.6	15.7	3.1
NR	7	14.4	16.1	1.7
SR	7	14.5	17.1	2.6
Totals		269.6	313.8	44.2
Mean		14.2	16.5	2.33
σ				1.01
σ_m				0.23

Rate of Eruption

In order to measure tooth eruption, 1 hole was drilled each week with an 800 L tapering fissure bur in the distal surface of each upper incisor tooth at the cemento-enamel junction even with the gingival margin. For this procedure, the animals were anesthetized. The eruption distance for the week was estab-

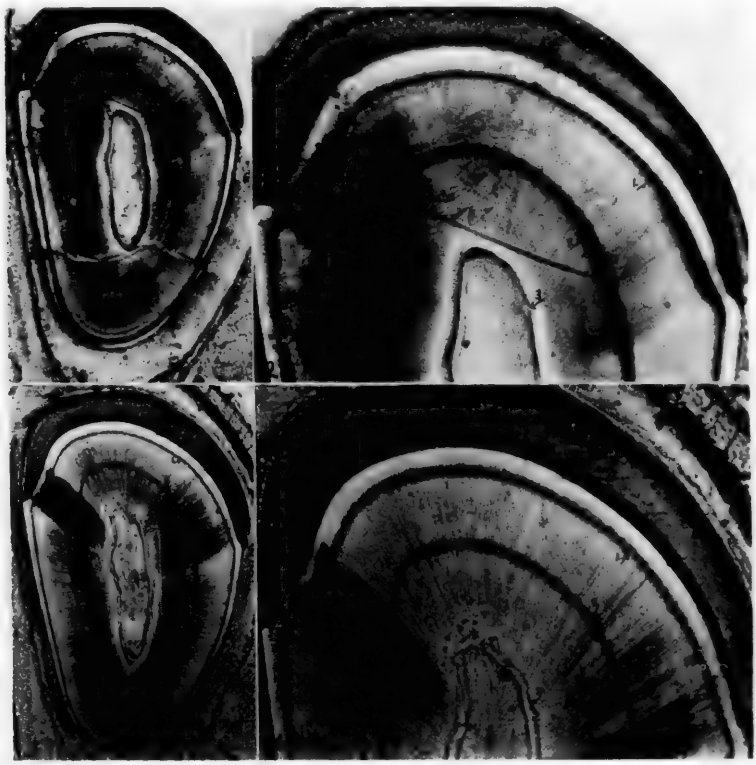

FIG. 1. Rat NL, Cage 2. Untreated animal. Shows 3 black rings in cross-section of incisor. These are "alizarin" red lines seen in original section. There was gain of 0.6 micra per day in dentin apposition rate in experimental period (lines 2 to 3) over control period (lines 1 to 2). × 56 (orig. mag.)

FIG. 2. High power of *fig. 1*. × 130 (orig. mag.)

FIG. 3. Rat NL, Cage 7. Treated animal. Alizarin rings show gain of 3.1 micra per day in dentin apposition rate. × 56 (orig. mag.)

FIG. 4. High power of *fig. 2*. × 130 (orig. mag.) Dentin structure appears normal.

lished by marking off with a divider the distance between the hole in the tooth and the free gingival margin, and measuring this distance by means of a micrometer. The process was repeated each week.

Results. Considerable variation (216 to 285 micra daily) was found in eruption rates of the incisor teeth in all the animals. In the treated series, the rates

TABLE III

Bone apposition rate

RAT	CAGE	CONTROL PERIOD	EXPERIMENTAL PERIOD	DIFFERENCE	DIFFERENCE
			Untreated animals		
		micra/day	*micra/day*	*micra/day*	%
Plain	2	6.06	5.82	−0.24	−4.0
NL	2	5.25	5.21	−0.04	−0.8
NR	2	2.93	2.46	−0.47	−16.0
SR	2	6.97	7.07	0.10	1.4
Plain	4	2.00	1.75	−0.25	−12.5
NL	4	6.00	6.14	0.14	2.3
NR	4	5.10	4.67	−0.43	−8.4
SR	4	3.25	2.82	−0.43	−13.2
Plain	8	4.78	4.92	0.14	2.9
NL	8	—	—	—	—
NR	8	7.36	6.35	−1.01	−13.7
SR	8	4.42	3.94	−0.48	−10.9
Totals		54.12	51.15	−2.97	−72.9
Mean		4.92	4.65	−0.27	−06.6
σ					0.33
σ_m					0.10
			Treated animals		
Plain	1	5.50	8.75	3.25	59.1
NL	1	5.35	6.93	1.58	29.5
NR	1	6.14	7.97	1.83	29.8
SR	1	3.57	5.15	1.58	44.3
Plain	3	3.29	4.23	0.94	28.6
NL	3	—	—	—	—
NR	3	4.21	6.11	1.90	45.1
SR	3	4.79	7.32	2.53	52.8
Plain	5	3.00	3.57	0.57	19.0
NL	5	2.57	3.93	1.36	52.9
NR	5	3.14	4.86	1.72	54.8
SR	5	4.32	5.50	1.18	27.3
Plain	6	3.48	5.04	1.56	44.8
NL	6	6.93	10.40	3.47	50.1
NR	6	—	—	—	—
SR	6	4.75	5.79	1.04	21.9
Plain	7	3.54	3.29	−0.25	−7.1
NL	7	5.28	6.21	0.93	17.6
NR	7	4.68	6.22	1.54	32.9
SR	7	4.00	4.57	0.57	14.2
Totals		78.54	105.84	27.30	617.6
Mean		4.36	5.88	1.52	34.3
σ					0.88
σ_m					0.21

of eruption in the upper incisors ranged from 214 to 280 micra daily, with the median about 250 micra. Statistical analysis showed no significant effect on eruption rate as a result of vitamin D administration (Tables V and VI).

Skeletal Calcification

Skeletal calcification was studied by the radiographic method. The animals were placed in a prone position, 2 untreated or 2 treated rats on each film. The whole animal was radiographed at a target distance of 5 feet. Radiographs of the mandibles and entire bodies were examined for increased density by comparing the treated with the untreated animals. No differences were observed between treated and untreated animals.

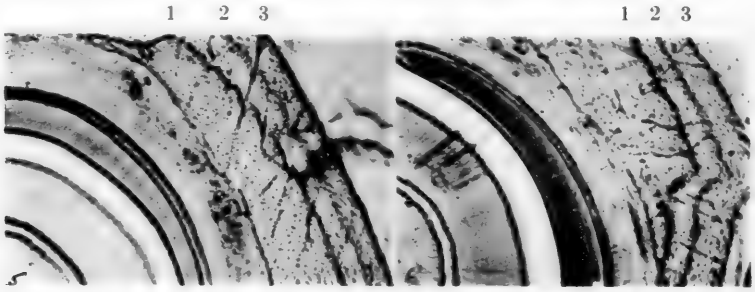

FIG. 5. Rat NL, Cage 2. Untreated animal. Alizarin lines in bone. Lines 1 to 2 constitute control period; Lines 3 to 4 experimental period. There was loss of .08% in daily rate of bone apposition. × 116 (orig. mag.)

FIG. 6. Rat NL, Cage 7. Treated animal. There was gain of 17.6% in daily rate of bone apposition in experimental period over control period. × 116 (orig. mag.)

Pulp Stones

The mandibles were removed, split in half, debrided of all soft tissue, and radiographed individually, at a target distance of 3 feet, for the detection of pulp stones. Molars and incisors were also examined radiographically. No pulp stones were observed.

Estrus Cycle

Daily vaginal smears were taken on all animals (6, 7, 8). The estrus cycle was found to be unaltered during the experimental period when compared with the control period.

Toxicity

The absence of toxicity in the treated group was judged by such general criteria as: constant weight increase, continued good appetite, silkiness of coat, and activity as contrasted with an earlier test group which received ten times the amount of Ertron and showed a definite toxicity as characterized by weight loss, lethargy, anorexia, roughness of coat, and increased irritability. None of the

TABLE IV

Calcification rhythm

RAT	CAGE	CONTROL PERIOD	EXPERIMENTAL PERIOD	INCREASE
Untreated animals				
		micra/day	*micra/day*	*micra/day*
Plain	2	12.6	15.0	2.4
NL	2	15.7	16.9	1.2
NR	2	13.9	15.0	1.1
SR	2	12.2	14.7	2.5
Plain	4	12.7	16.4	3.7
NL	4	15.5	17.8	2.3
NR	4	15.5	18.0	2.5
SR	4	15.4	15.5	0.1
Plain	8	14.3	14.1	−0.2
NL	8	15.6	19.8	4.2
NR	8	14.9	17.1	2.2
SR	8	13.9	13.5	−0.4
Totals		172.2	193.8	21.6
Mean		14.4	16.2	1.80
σ				1.41
σ_m				.41
Treated animals				
Plain	1	14.3	15.3	1.0
NL	1	11.8	16.8	5.0
NR	1	11.3	16.9	5.6
SR	1	11.5	10.0	−1.5
Plain	3	13.5	17.8	4.3
NL	3	13.7	14.1	0.4
NR	3	12.6	17.5	4.9
SR	3	12.5	15.0	2.5
Plain	5	17.9	17.5	−0.4
NL	5	13.5	14.6	1.1
NR	5	13.7	15.0	1.3
SR	5	—	—	—
Plain	6	17.1	15.4	−1.7
NL	6	15.9	14.3	−1.6
NR	6	14.3	14.9	0.6
SR	6	13.9	12.5	−1.4
Plain	7	16.9	15.4	−1.5
NL	7	14.1	15.5	1.4
NR	7	15.5	15.9	0.4
SR	7	12.8	15.3	2.5
Totals		266.8	289.7	22.9
Mean		14.0	15.2	1.21
σ				2.32
σ_m				.53

TABLE V

Eruption rate

RAT	CAGE	CONTROL PERIOD	EXPERIMENTAL PERIOD	DIFFERENCE
Untreated animals				
		micra/day	*micra/day*	*micra/day*
Plain	2	251	220	−31
NL	2	251	253	2
NR	2	251	236	−15
SR	2	258	241	−17
Plain	4	272	220	−52
NL	4	245	236	−9
NR	4	251	244	−7
SR	4	251	214	−37
Plain	8	285	272	−13
NL	8	248	251	3
NR	8	230	244	14
SR	8	244	244	0
Totals		3037	2875	−162
Mean		253	239	−14
σ				18
σ_m				5.2
Treated animals				
Plain	1	265	261	−4
NL	1	251	245	−6
NR	1	265	261	−4
SR	1	251	280	29
Plain	3	251	249	−2
NL	3	258	238	−20
NR	3	258	216	−42
SR	3	258	238	−20
Plain	5	251	242	−9
NL	5	223	220	−3
NR	5	272	216	−56
SR	5	251	186	−65
Plain	6	258	208	−50
NL	6	244	238	−6
NR	6	251	248	−3
SR	6	244	220	−24
Plain	7	251	214	−37
NL	7	251	236	−15
NR	7	224	253	29
SR	7	258	253	−5
Totals		5035	4722	−313
Mean		252	236	−16
σ				24
σ_m				5.4

enumerated toxic symptoms were exhibited. The rats appeared entirely healthy and continued to increase in weight (Table I).

TABLE VI

Significance tests for differences in mean increase between treated and untreated animals

	DIFFERENCE IN MEAN INCREASES	S.E. OF DIFFERENCE IN MEAN INCREASE	CRITICAL RATIO
Dentin apposition	1.39	0.33	4.2*
Bone apposition	1.79	0.23	7.8**
Calcification rhythm	0.59	0.67	0.88†
Eruption	−2.2	7.5	0.29††

* The difference between the untreated and treated animals in average increase in dentin apposition is statistically significant. The probability that a difference as great as the one observed or greater would arise solely through chance or errors of random sampling, is less than one in a thousand.

** The difference between the untreated and treated animals in average increase in bone apposition is statistically significant. The probability that a difference as great as the one observed or greater would arise solely through chance or errors of random sampling is less than one in a million.

† The difference between the untreated and treated animals in average increase in calcification rhythm is not statistically significant. A difference as large as the one observed or larger would arise about 40% of the time solely through chance or errors of random sampling.

†† The difference between the untreated and treated animals in average increase in eruption rate is not statistically significant. A difference as large as the one observed and larger would arise about 80% of the time solely through chance or errors of random sampling.

TABLE VII

Vitamin D dosage of rheumatoid arthritic patients*

PATIENT	AGE	SEX	TOTAL UNIT DOSAGE	TOTAL DURATION PERIOD (DAYS BETWEEN RADIOGRAPHS)
J. W.	33	Female	41,700,000	153
H. S.	47	Female	68,250,000	231
C. P.	71	Female	29,150,000	103
V. B.	55	Female	34,550,000	121
E. W.	29	Female	62,250,000	218
I. R.	61	Female	48,950,000	169
L. T.	48	Male	63,750,000	219
I. F.	48	Female	67,250,000	230

Note: In 3 cases the first set of radiographs was taken a few days after instituting therapy.

* Ertron.

CLINICAL STUDY

Dental radiographs of 8 patients suffering from rheumatoid arthritis were taken before and after daily therapy of approximately 300,000 units of vitamin D (Ertron) was instituted. The pictures were compared for a possible difference in pulp stone formation. No appreciable change was seen (Table VII).

SUMMARY

1. Dentin apposition appeared to be significantly greater in the rats receiving vitamin D (Ertron) than in the untreated group.

2. No difference was observed in the calcification rhythm of the dentin of the lower incisors of the animals fed vitamin D as compared with the untreated group.

3. A significant increase in bone formation occurred during the period when the specific vitamin D preparation was fed as compared to the amount of apposition during the control period. No such increase occurred in the untreated animals.

4. The rate of tooth eruption was not altered significantly in the animals receiving vitamin D as compared with the untreated animals.

5. The skeletal calcification, as observed by comparing the radiographs, was not significantly different in the treated and untreated groups.

6. No pulp stones were seen (in radiographs) in any of the animals, even after the massive vitamin D administration. No appreciable change in pulp stone formation was detected (in radiographs) in 8 humans receiving approximately 300,000 units of vitamin D (Ertron) daily.

7. The daily administration of massive doses of Ertron did not significantly alter the estrus cycle of the rats during the experimental period.

8. Daily massive doses of vitamin D (Ertron) were administered without signs of toxicity.

The authors wish to express their appreciation to Drs. Bion R. East and John W. Fertig of the DeLamar Institute of Public Health, Columbia University, College of Physicians and Surgeons, for their assistance in the preparation of the statistical data.

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STUDIES ON THE INCIDENCE AND CAUSE OF DENTAL DEFECTS IN CHILDREN[1]

V. Freedom from Caries

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Children free from caries have been reported in all parts of the world, regardless of the locality, the food consumed or the habits of oral hygiene. An accurate report on children unaffected by this disease is rendered extremely difficult by the lamentable fact that teeth may be filled even when non-carious. Accounts coming from communities where such a procedure is in vogue must, therefore be considered as relative. The number of those free from caries is, no doubt, reduced where such trends dominate. Thus the obstacles to a solution of the problem of dental caries are greatly increased.

A closer approach to a study of children free from caries can be attained by considering the primary causes and the contributory factors declared to be responsible for dental decay. Miller's (1) chemico-parasitic theory gains additional strength many years after its promulgation from Belding and Belding (2, 3), even though they regard it as "incomplete". They are thoroughly convinced that "acids and acids alone" are the exciting factors producing enamel disintegration. It is their belief that a tooth, no matter how poorly developed or how badly fissured, will not become carious if its owner will refrain from consuming fermentable food substances, even though no attention is paid to oral hygiene. The relation of lactic acid to the production of caries is emphasized by Bodecker (4), who believes that dental decay could be prevented by the discovery of a method to "chemically bind this acid" previous to its deleterious effect on the mineral salts forming the enamel. Fosdick's (5, 6) work seems to indicate that interference with the enzyme system (which ferments carbohydrate food particles) results in a decrease in the amount of acid produced through fermentation. Such interference can be effected through a substantial reduction in the intake of free sugars and through thorough cleansing of the teeth and mouth soon after meals.

Bibby and Hine (7) examined 44 smears obtained from different carious cavities in 44 mouths and found, at the base of each cavity, a complex bacterial flora. The presence of a higher percentage of gram negative than gram positive bacilli, to which group the *Lactobacillus acidophilus* belongs, cast a doubt on the importance of the latter in the production of caries. However, according to Reed (8), a mouth free from caries is practically free from these microorganisms. Starch and bacteria, he finds, are the direct causes of dental decay. Immunity

[1] Received for publication, June 10, 1943.

to this disease would, therefore, be obtained by eliminating one of these factors. A diminished carbohydrate consumption will effect a reduction in the *Bacillus acidophilus* count per cubic centimeter of saliva. Reed's position is substantiated in a large measure by the earlier work of Bunting (9) and others, and by the later studies of Robinson (10, 10a).

It was in 1929 that Bodecker (4) presented the theory that the fundamental factor in the resistance of the teeth to caries is the "dental lymph". The dental lymph, he believed, is concerned in the neutralization of lactic acid it encounters in its passage through the enamel and dentin. The key to normal dental lymph is a healthy condition of the individual. Taking these claims into consideration, the question arises: if normal dental lymph is found in healthy individuals, how can the prevalence of caries in many such subjects be explained? Then again, if normal dental lymph is not present in frail or sick individuals, why is it that many such persons are free from caries? Obviously this interesting theory requires proof to make it acceptable.

Rural districts appear, in some instances, to furnish higher numbers of pupils immune to dental caries. For example, King (11), in his examination of 1376 English children inhabiting the Island of Lewis and the adjoining mainland county of Ross-Shire, found that 22.1% of those living in rural districts were free from caries, while only 2% of those residing in the towns and a fraction over 6% of those inhabiting the mainland showed freedom from the disease. Confirmation of these findings is contributed by Mansbach (12), who in his investigation of large numbers of children from rural and urban districts of Palestine noted better teeth in the rural contingents. Another group of pupils to be considered are the Indian school children examined by Arnim, Aberle and Pitney (13). Of 204 examined, 13% were free from caries. These children inhabited rural communities, 6 Rio Grande pueblo villages and 3 Hopi villages in Arizona. Children of a number of other semi-urban and urban regions show an increased susceptibility to dental decay. Such instances are furnished by Clements and Kirkpatrick (14), McCall (15), Day and Sedwick (16), and others.

If we explore the relation of race and immunity to dental caries, we discover that in Atlanta, Georgia, an urban community, Sterling (17) found 31% of 5000 negro children free from caries. In the city of Bridgeport, Connecticut, 18% of 56 American negro children examined by Coon and Rosenthal (18) were free from the disease. Indeed the contrast is sharp. In my own group of 1882 negro children to be reported on here, 411 (22.5%) were caries-free. In an additional investigation which will form the main substance of one of my future reports, 31% of a total number of 899 negro pupils revealed a complete absence of tooth decay.

No discussion on children free from caries can be complete without a consideration of the subject of heredity. Observations by Le Norcy (19, 20), Kugelmass (21), Mabalay (22), Day and Sedwick (16), Harris (23), and others led them to suggest that heredity acts as a predisposing cause. On the other hand, Belding and Belding (24), Gilmour (25), Shaw (26), and also Leonard (27) found no reason to believe in the theory of hereditary tendency to dental decay. Wallace (28) stated that mutilations are "not inherited". "In its initial stage caries

is a mutilation produced by the action of acid on the inorganic enamel." Impartial students of the problem have come to realize that the theory of heredity presents serious difficulties. The suggestion that the size and shape of the teeth are inherited does not alter the fact that caries is an acquired abnormality. Many years ago Weismann (29) proved that acquired characteristics are not inherited.

DATA

Additional interest to the subject of children free from caries will be contributed by a presentation of findings pertaining to a group of 9,269 school children from Newark, New Jersey. Their ages ranged from 6 to 16 years; their socio-economic circumstances varied; 20 nationalities were included.

Of the total number of pupils inspected, 1,148 were free from caries, 592 boys and 556 girls. Of those who resisted dental decay, 411 were negroes, 188 boys and 223 girls. Pupils were considered to be free from caries of the deciduous teeth when the full complement of these organs was present, when no dental repair had ever been attempted on them, and when no indication of dental decay was evidenced. Children were declared free from caries of the permanent teeth when these showed no evidence of ever having been repaired and when no dental decay was present at the time of examination, and no permanent teeth had been previously extracted. Students were classed as free from caries of the mixed dentition when the deciduous and permanent teeth present showed no evidence of decay or of repair attempted on them, and where no permanent teeth had been extracted. The mouth mirror, explorer, college pliers, scalers and cotton pellets were the agents employed in a most careful examination. X-ray examinations were not included. A tooth was declared carious if decayed at the time of examination.

The group of pupils was divided into 2 subgroups: first, those who belonged to a socio-economic level above the average; second, those whose economic status was rather poor. One group of 116 pupils comprised a mixed socio-economic contingent.

The percentage of negro girls without caries was above that of the white girls (25.6% negroes and 9.2% white girls). More negro boys than white boys also were free from the disease (negroes, 19.6%; white boys, 10.5%). We are, therefore, confronted by the fact that there is undoubtedly a greater resistance to caries in the negro pupils. The reason for this is at present obscure. Such factors as diet and attention to oral hygiene were fully considered but furnished no conclusive answer. Studies on this subject are being continued. Although the percentage of negro girls free from caries exceeded that of the negro boys, the percentage of white boys without caries was slightly higher than that of the white girls. The influence of sex on freedom from caries, while rather definite in the colored group, appeared inconclusive in the white children. More evidence on this aspect of the question is necessary.

COMMENT

It would seem at first that social status might play a part in the freedom of children from caries, but such influence was difficult to conclude from these

studies. While it is true that one of the highest percentages was registered in a group of 109 pupils with a rather low standard of living, the fact must be noted that another group of 136 children, of a similar stratum but residing in another section of the city, showed a much smaller percentage free from the inroads of the disease (School No. 9, 17%; School No. 8, 6.6%). Similar differences were observed in the highest economic levels. The percentage of children free from caries was much higher in one group of 1,678 pupils than in a group of 1,362

TABLE I

Freedom from caries in a group of white and negro children of both sexes

	WHITE		COLORED	
	Boys	Girls	Boys	Girls
No. pupils examined	3845	3602	951	871
No. free from caries	404	333	188	223
% free from caries	10.5%	9.2%	19.6%	25.6%

TABLE II

Freedom from caries and social status

	NUMBER OF PUPILS EXAMINED	NUMBER FREE FROM CARIES	% FREE FROM CARIES
*School No. 1	1362	133	9.7
*School No. 2	1678	215	12.8
*School No. 3	243	41	17.8
*School No. 4	1021	131	12.8
*School No. 5	350	57	16.3
**School No. 6	116	7	6.03
***School No. 7	104	5	4.8
***School No. 8	136	9	6.6
***School No. 9	109	19	17.4
***School No. 10	1283	89	6.9
***School No. 11	44	5	11.3
***School No. 12	854	106	12.4
***School No. 13	1190	251	21
***School No. 14	779	80	10.3

* Pupils belonging to a "better" economic stratum.

** Group affected with various bone diseases (mixed social strata).

*** Pupils belonging to a rather low economic level.

pupils residing in a nearby district and attending a nearby school (School No. 2, 12.8%; School No. 1, 9.7%). Further conclusions can be drawn from the fact that 12 per cent of the 4,654 pupils, belonging to a higher socio-economic level were declared free from caries, and also that the 4,615 children comprising the lower socio-economic group showed the same percentage (12%). These findings are a striking evidence that social status cannot furnish a clue to the problem of dental caries.

That disease may be a factor in increasing susceptibility to caries was suggested by a group of 116 pupils affected with various bone diseases and of whom only 6.03% were free from caries. Nevertheless, it is difficult to admit that Bodecker's theory of "normal dental lymph" is applicable here, for some groups of healthy subjects showed quite similar and even smaller percentages of children free from caries.

Mention has been made of the fact that, according to some investigators (King [11], McCall [15] and others), rural districts furnish larger numbers of pupils free from caries. While this is true in some localities, the situation can hardly be considered as general. For instance, my investigations showed that in one group of pupils (School No. 3) in an urban community, the percentage of freedom from caries was higher than that discovered by Arnim, Aberle and Pitney in the group of Indian children they studied (Arnim, Aberle and Pitney, 13%; Brucker, 17.8%). Of course, comparisons between urban districts reveal differences in the number of pupils free from dental decay, but this is also the case when rural communities are compared. I am convinced that neither city life nor country existence are deciding factors in freedom from caries.

The subject of heredity has already been mentioned. The opinions of Le Norcy, Kugelmass, Day and Sedwick, and others have received a deserved share of attention. However important they may be, they cannot alter the fact that caries is an acquired injury. Weismann's (30) studies on 22 generations of mice whose tails he amputated but who continued to give birth to progeny with full-length tails, have supplied a contribution to science which must always be considered when the hereditary transmission of bodily mutilation is deliberated. Scientists who have repeated Weismann's experiments agree that he was correct, and their work convinces one that such acquired characteristic cannot be transmitted.

The relation of nationality to freedom from caries was difficult to ascertain from these investigations. Intimations of a possible influence were noted in one or two groups but generally appeared rather inconclusive. What I found, however, was that the similarity in the percentage of caries-free children in the various nationality groups was limited to the white pupils and did not apply to the negroes. In the latter, as previously explained, freedom from caries was definitely more prevalent. More studies are being undertaken in this direction.

Furthermore, individuals free from the disease in groups of children with hypoplasia as well as among those with malocclusion were noted. An explanation for this may be found in the fact that hypoplastic teeth are not, as a rule, attacked by caries. Freedom from dental decay in some cases of malocclusion may perhaps suggest that where there is resistance to caries it may fail to develop regardless of any influences whatsoever. Additional studies along this line are needed.

If comparison is made with other urban communities, it will be noted that the Newark group supplied a larger number of pupils free from caries than that reported by investigators in different cities of the United States (Chicago (31), Rochester, N. Y. (16)), some regions of England, Australia, South Africa, etc.

Arnim, Aberle and Pitney's rural territory (New Mexico and Arizona) furnished a smaller number of children free from caries than a similar group in Newark, an urban district.

It must be recognized that geographical and climatic conditions seem to play no certain part in promoting freedom from the disease. For example, Atlanta, Ga., a southern urban community with a larger number of sunshine hours per year than Newark, N. J., an urban but eastern community, had a similar percentage of negro children free from caries (Atlanta, 31%; Newark, 31%). The salubrious climate of New South Wales, Australia, with an average of only 26 days in the year devoid of sunshine, appears to confer freedom from caries to a limited degree, Clements and Kirkpatrick (14) reporting a fraction over 7% of a group of children examined in sections of that territory free from the disease. The city of Johannesburg, South Africa, is favored with a high altitude and a considerable amount of sunshine, yet less than 7% of a group of children examined in that community was caries-free (32). Newark, N. J., with an average of over 200 days in the year devoid of sunshine, furnished a larger number of pupils free from caries (Table I).

In view of these illustrations and others which might be considered, we must admit that freedom from caries is an issue difficult to explain. Possibly the influence of the endocrines is a matter which cannot be ignored.

SUMMARY

The extent of freedom from caries is reported for a group of pupils which has been thoroughly investigated. A report on children free from caries is a difficult task in communities where prophylactic odontotomy is in vogue for, under the circumstances, the number of individuals free of filled teeth is no doubt reduced.

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THE CITRATE CONTENT OF WHOLE TEETH, DENTIN AND ENAMEL[1]

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For a long time it has been appreciated that citrate is a constituent of milk and urine. With the development of more accurate and specific methods (1, 2) for the determination of citrate, this ion has been recognized as a constituent of many tissues and body fluids. The occurrence of citrate in the animal body has been reviewed by Smith and Orten (3). Scherstén (4) has shown that there is quite a large amount of citrate in semen and in the secretion of the seminal vesicles, the concentration in semen being as high as 410 mg. per 100 cc. and in the secretion of the seminal vesicles as much as 633 mg. per 100 gm. which is comparable to the amounts of citrate found in milk and in alkaline urine. These observations have been extended by Huggins and Neal (5) who found in 9 samples of human prostatic fluid, citrate concentrations ranging from 480 to 2688 mg. per 100 cc. Dickens (6) discovered that bone contains a very large amount of citrate, the concentration ranging from 272 mg. per 100 gm. up to as high as 1660 mg. per 100 gm. in dried powdered bone. This investigator isolated both the pentabromoacetone derivative of citric acid and pure citric acid from bone meal. The present report describes studies made of the occurrence of citrate in whole teeth and in separated enamel and dentin.

EXPERIMENTAL

Twenty sound human teeth of different varieties were cleaned of all organic debris and were dried in an oven at 100°C. These dried teeth were then ground in a steel mortar so that the material had the consistency of a powder. This powder was analyzed for citrate according to the method described below. Three large canine teeth from a dog were treated in a similar manner.

To determine citrate on the pooled whole teeth a 50 mg. sample was suspended in 50 cc. of 10% trichloroacetic acid to which 3 cc. of 50% sulfuric acid were added. This mixture was heated to boiling with a resultant solution of the ground tooth powder, and the solution was then analyzed for citrate by the colorimetric method of Pucher, Sherman and Vickery (2).

Separation of enamel and dentin was accomplished by the method of Brekhus and Armstrong (7). The separated dentin and enamel were dried and analyzed by the same method used for the whole ground teeth.

The results of the citrate analyses on the teeth are summarized in Table I.

[1] Received for publication September 3, 1943.

It will be seen that both human teeth and dog teeth contain somewhat more than ½% of citrate expressed in terms of citric acid. These figures are of the same order of magnitude as those obtained by Dickens (6) on bone. Separate analyses of enamel and dentin indicate that these two different portions of tooth differ quite markedly in their citrate content. This difference calls to mind the different embryological origin of the dentin and enamel. Both embryologically and chemically the dentin is more similar to the bone, and in citrate content also the dentin resembles bone.

Attempts were made to determine whether the citrate of whole ground teeth is in a soluble form. The citrate was completely insoluble in both petroleum ether and diethyl ether. Extraction of the powdered whole tooth with hot or cold water succeeded in dissolving a small amount of the citrate but the apparent solubility was only a small fraction of that of calcium citrate. Either cold water (extraction period of 4 days) or hot water (extraction period of 2 hours) dissolved from 10 to 20% of the citrate present in the ground tooth. The

TABLE I

Citrate content of teeth

TYPE OF SAMPLE	MG. PER 100 GM. (CITRIC ACID EQUIVALENT)
Whole human teeth	680
Human dentin	800
Human enamel	90
Whole dog teeth	510
Dog dentin	540
Dog enamel	110

nature of the citrate in teeth is thus quite obscure. The functional significance of the citrate is even more uncertain.

SUMMARY

Pooled dry human teeth contained 680 mg. of citrate (expressed as citric acid) per 100 gm. and pooled dog teeth contained 540 mg. of citrate per 100 gm. Separate analyses of dentin and enamel indicated that dentin has from 5 to 8 times as much citrate as does enamel.

The author is indebted to Miss Esther Gimp for valuable technical assistance.

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A STRUCTURELESS RECURRENT DEPOSIT ON TEETH[1]

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Investigations on the nature of accumulations on the surfaces of erupted teeth have usually indicated that these deposits are composed in part of oral bacteria, and that they are capable of reforming on the teeth within a few days after their removal by abrasives. The colorless pellicle of the enamel cuticle (Nasmyth's membrane) is an exception to both of these generalizations, in that it is devoid of microorganisms and is usually considered to be incapable of further development after the teeth have erupted (1).

This paper is concerned with a deposit which is similar to the enamel cuticle in its freedom from microorganisms, but which recurs after removal like the majority of dental accumulations. This recurrent pellicle has been termed brown pellicle, because its most obvious macroscopic property is its brown pigmentation, and its most noteworthy microscopic characteristic is its freedom from structure. There seems to be no record of observations in the dental literature on a deposit with these characteristics, and while accumulations have been described that are similar in some respects, there is not sufficient evidence concerning them to identify them as brown pellicle.

GENERAL PROPERTIES OF BROWN PELLICLE

The pellicle develops upon the labial and lingual surfaces of the teeth of some of the persons who do not use abrasives in their daily oral hygiene. It may be noted macroscopically within a week on certain individuals as a decrease in the luster of enamel. A week later the film itself can be recognized as a pigmented pellicle that is distributed preferentially on the anterior teeth and labial surfaces (*fig. 1*). The color is usually brown or gray, but occasionally light brown or dark gray deposits are seen. Sometimes the central third of the labial surface is most heavily coated and frequently the cervical third is not covered. When the pellicle develops over a considerable period of time, or upon a tooth which is protected from brushing by its malposition in the arch, it may be laid down in a reticulated pattern. It tends to be reticulated on silicate fillings, and rarely accumulates on porcelain restorations.

In every case tested, the deposit could be removed by 1 or 2 brushings with a dentifrice grade calcium phosphate or carbonate (*fig. 1*), but not by brushing with a dry or with a moistened brush. Soap and several synthetic detergents

[1] Read at the 20th General Meeting of the International Association for Dental Research, Chicago, Illinois, March 14, 1942. (*J. D. Res.*, **21**: 322 (1942). Received for publication Sept. 29, 1943.

were tried out in abrasive-free dentifrices in the daily oral hygiene, but were unsuccessful in preventing pellicle formation. The rate of formation of brown pellicle has remained practically constant for several subjects over a period of 3 years, as determined by frequent periods of formation and removal. Microscopic examination of the teeth of these subjects has shown that the enamel surface has not been affected in any observable way.

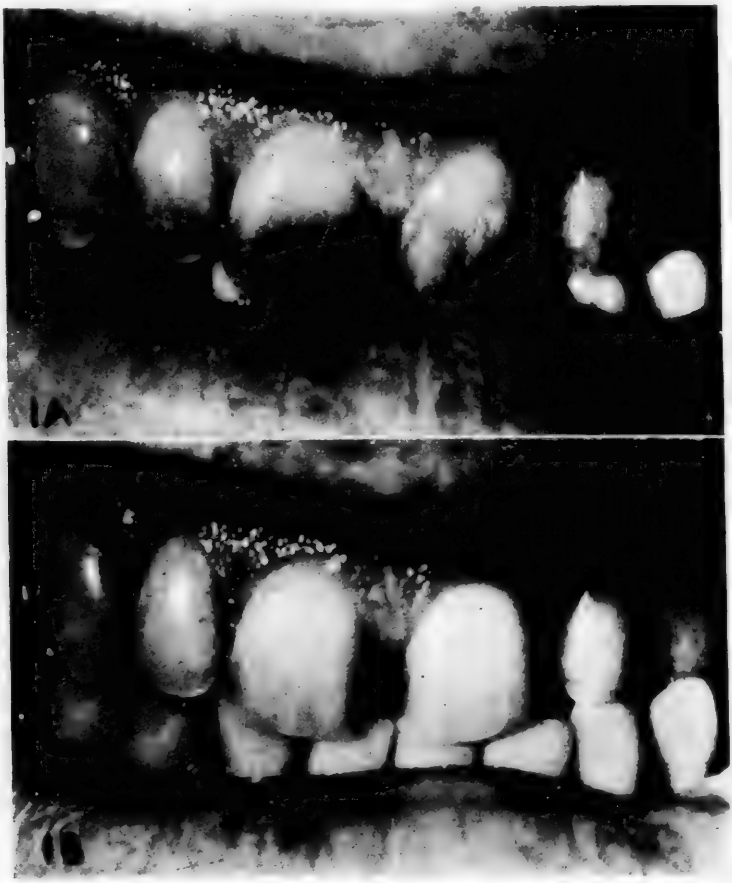

FIG. 1A. Appearance of brown pellicle on teeth.

FIG. 1B. Appearance of same teeth after removal of pellicle by single brushing with dentifrice grade $CaHPO_4$.

The quantities of pellicle available on the teeth of our subjects were so small that very few chemical tests could be tried. A 2 week's deposition of brown pellicle on one subject provided about 0.01 mg. of scrapings. However, positive Millon's and xanthoproteic tests were obtained with the aid of a microscope, and when the pellicle was burned, the smell of burning hair was noted. A micro-ash attempted with only 0.03 mg. of sample merely indicated that the ash per-

centage was low, but this and the other findings suggested that the pellicle consists chiefly of protein.

MICROSCOPIC OBSERVATIONS

For detecting the pellicle macroscopically it was found best to make the examinations after the enamel surface had been exposed at 1 or 2 spots by scraping, since the contrast between the enamel and the film disclosed its presence. In

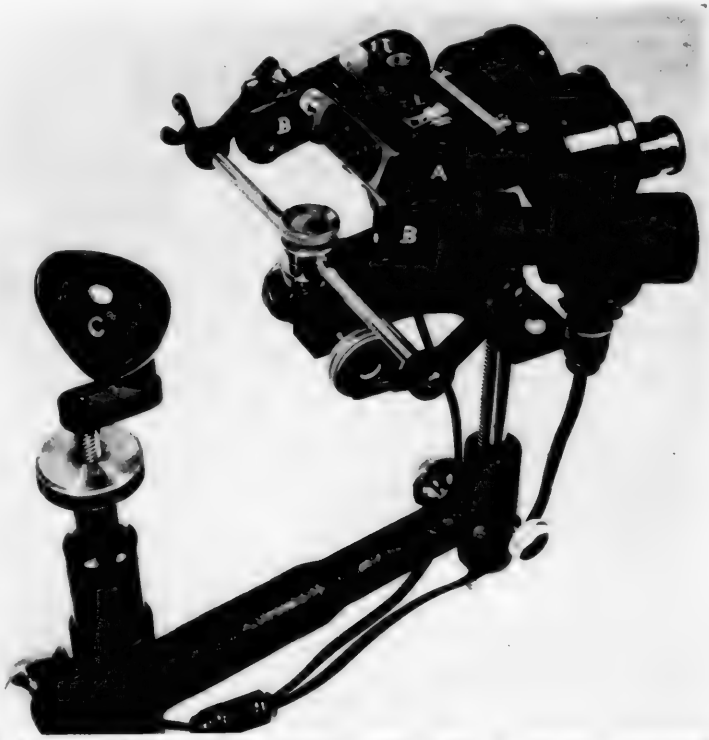

FIG. 2. Apparatus for examining anterior teeth in order to estimate amounts of brown pellicle present. Binocular microscope, A, and Nicholas Illuminators, B, B, are brought into focus on teeth of subject whose chin is placed at C.

the early stages, however, the pellicle was so thin that it could not be identified readily except by examination of the teeth with a microscope. The apparatus used for such examinations (*fig. 2*) consisted of a binocular microscope, special lighting equipment, and a chin rest arranged to permit the microscope and lights to be focussed steadily on each anterior tooth in a subject's mouth. The microscope was a Bausch and Lomb binocular type with 15 times magnification, and the light was furnished by 2 Nicholas illuminators. Such equipment made it easy to distinguish between the brown pellicle and calculus or materia alba. The relative amount of pellicle present on the teeth could be estimated by

assigning a score ranging from 0 to 4 to each of the 8 incisors, and by using the sum of such scores as a measure of the total quantity of pellicle.

Samples of the film were scraped off for study after materia alba had been removed by brushing the teeth with a detergent solution. For microscopic observation the scrapings were suspended in a drop of water, spread on a slide, dried, and fixed by passing through a flame.

Fig. 3 is a photomicrograph of scrapings stained by a modified Gram's method (2). The greater part of the deposit consisted of a structureless, faintly Gram-positive film that appeared to be free of microorganisms.

Frequently associated with it were strongly Gram-negative granular clumps, which were further differentiated from the pellicle by the contrasts in their reactions to toluidine blue, thionine, haemotoxylin and muchematin stains. When centrifuged salivary mucin was allowed to evaporate on a slide, fixed by heat, and scraped loose in spots, it absorbed these stains more in the manner of

FIG. 3. Faintly Gram-positive pellicle and Gram-negative clumps, 250 times magnification, stained by a modified Gram's method (2).

the pellicle than of the clumps, yet it differed distinctly from the pellicle in that it contained enmeshed microorganisms.

SOLUBILITY TESTS

The solubility of the film was determined on pellicle scrapings in capillary tubes, using photomicrographs to record gross changes in the precipitate volume. The film was scraped from the anterior teeth with a silver scraper, and transferred to the spot plate in a drop of water. The drop suspending the precipitate was picked up by capillarity into a tube 1 mm. in diameter, into one end of which the pellicle was centrifuged after the same end had been sealed in a flame.

The appearance and volume of the precipitate were recorded by photograph with an Argus C^3 Camera mounted on one eyepiece of a Bausch and Lomb wide field microscope. In order to make the contents of the capillary tube more easily visible, the tube was immersed in a liquid (tributyl phosphate) having a refractive index similar to that of glass, and covered with a microscope coverslip.

A capillary pipette was prepared by drawing out one end of a 1-mm. tube and sealing the other end. This was warmed with flame and the tip introduced into the water over the sample. The cooling of the pipette served to draw off the supernatant liquid. The reagent to be investigated was introduced into the capillary by heating the pipette in which it was was contained. The drop of reagent was stirred with the precipitate either by centrifuging it from end to end, or by agitating the capillary after a micro-bead of glass had been introduced.

TABLE I

Color changes and solubility of brown pellicle in various reagents

	28°C.		90°C.	
	Color change	Solubility	Color change	Solubility
Basic Solutions				
1 N and 0.1 N NaOH, 4% Na_2CO_3	None	Insol.	—	Dissolved
Concd. NH_4OH, 1% Na_3PO_4, 50% triethanolamine, diethanolamine, pyridine and morpholine	"	"	None	Insol.
Acidic Solutions				
HCl, concentrated	"	"	Black	Decomposed
HNO_3, 10%	Light orange	"	—	Dissolved
H_2SO_4, 10%	Red orange	"	Orange	Insol.
Glacial acetic acid, phenol & cresol	None	"	None	"
Protein Solvents				
10% and 1% Na_2S	"	"	—	Dissolved
10% sodium salicylate	"	"	Slight	Insol.
50% urea	"	"	None	"
Oxidizing and Reducing Agents				
5% Ca $(ClO)_2$	Bleached	"	Bleached	Decomposed
Oxalic acid	Orange	"	Orange	Insol.
Cellosolve, butyl cellosolve, diethylene glycol & tributyl phosphate	None	"	None	"

After the suspension had stood for about 1 hour, the precipitate was centrifuged to the end of the capillary and photographed to check any discernible change in its appearance or volume. A third photograph was taken after the sealed tube had been placed in a steam bath for about one hour at 90°C. A comparison of the photographs served to show whether any significant solution had taken place.

The studies on solubility are summarized in Table I. Apparently the pellicle is dissolved only by hot solutions that are capable of producing oxidation or

hydrolysis of the most resistant proteins. None of the reagents tested was able to bring about solution at room temperature, and these included solutions of urea and of sodium salicylate. Since dry gastric mucin dissolved readily in hot 50% urea to form a clear solution that did not become turbid on cooling or diluting, evidently the pellicle differs from mucin in its properties. Hydrolysis probably takes place when the pellicle dissolves in hot sodium sulfide or hydroxide, since hot ammonium hydroxide does not bring about solution. Hot hydrochloric acid is capable of hydrolyzing proteins and forming a black deposit of humin with some. It is interesting that a black precipitate was formed when the pellicle was treated with hot hydrochloric acid but there was no apparent decomposition when the pellicle was subjected to the nonhydrolytic glacial acetic acid.

The color of the pellicle was bleached readily by acids and by one oxidizing agent, without affecting the volume of material that remained. Oxalic, nitric and sulfuric acids produced some decolorization immediately upon contact with the film at room temperature, and 5% calcium hypochlorite produced instantaneous, complete decolorization. However, there was no bleaching action observed when 5% hydrogen peroxide was tested on the film *in vivo*.

These findings suggest that brown pellicle is composed for the most part of a resistant protein resembling keratin in its solubility, and that this protein is pigmented by a substance that is labile to acids and oxidizing agents.

COMMENT

The distinguishing characteristics of brown pellicle are its occurrence among some but not all of any group tested, its recurrency, its freedom from structure and microorganisms, its chemical inertness, and its resistance to removal by the brush alone. This particular combination of properties, however, would not likely be discovered unless the pellicle were observed on the teeth of susceptible persons using abrasive-free dentifrices, and it was because of recent usage of such products that this unique deposit came to be recognized.

A few investigators have observed deposits forming on teeth under conditions which should have produced brown pellicle, that is, when abrasives were omitted from daily oral hygiene. Thomssen (3) stated that only a part of the deposits on teeth are removed by soap-base liquid dentifrices, and Head (4) observed a brown precipitate on the teeth of persons using a special abrasive-free solution with their brushes. Both authors found that the deposits could readily be removed by brushing with abrasives, and while it is likely that what they saw was brown pellicle it cannot be proved because they made no further study of the accumulations. Howitt, Fleming and Simonton studied a subject who omitted brushing altogether and developed a pigmented film on his teeth (5). This deposit may have been brown pellicle in part but scrapings of the material contained considerable bacteria, and the authors did not actually describe any structureless membrane constituting the deposits which they observed.

If brown pellicle were observed in material obtained from extracted teeth, one would expect that it would be described as a structure-free, chemically resistant

protein deposit. For example, it might have been present in the plaques on the extracted teeth studied by Dobbs (6), who found a portion that was chemically resistant and a part, perhaps the same material, which was free of structure. Also there is a similarity between brown pellicle and brownin (7), the protein deposit which gives the brown coloration to mottled enamel. Like the pellicle, brownin has been described as being bacteria-free and resistant to alkali, and it similarly develops only on a part of the persons among groups which offer it opportunity to form (8, 9, 10). Furthermore, brownin develops within porous enamel where the abrasives commonly present in dentifrices could not possibly wear it away. And thus it likewise forms when abrasive action is absent.

The brown pellicle also seems to bear a close resemblance to the colorless pellicle of the enamel cuticle. Both are chemically inert protein deposits which possess a high degree of resistance to mechanical wear. Chase's description of Nasmyth's pellicle (enamel cuticle) is that it "is usually transparent, homogeneous, and slightly basophile, but may be overlaid by a thin layer of translucent, granular, acidophile substance" (11); this describes equally well the microscopic appearance of the pellicle and the clumps which are frequently associated with it. Such similarity is of course surprising in view of the fact that the brown pellicle is definitely recurrent on erupted teeth under the proper conditions, whereas the enamel cuticle is not supposed to be capable of reforming on the teeth after their eruption. It seems to the author that the similarity between the two pellicles is more convincing than the evidence which supports the hypothesis of the non-recurrent nature of the enamel cuticle, and perhaps it reopens the question as to how the enamel cuticle is formed and whether it is the same as the brown pellicle.

The similarities among brown pellicle and brownin, Nasmyth's pellicle and Dobb's pellicle do not afford any clues as to what are the precursors of the film with which the present study is concerned. Because brown pellicle seldom develops upon porcelain restorations, it might be inferred that a tooth enamel surface is necessary to its formation. Yet the use of extracted teeth did not result in the development of any pellicle when tested *in vitro* by means of repeated drying of saliva on teeth over a period of 2 weeks. Perhaps there is a labile agent which is rapidly lost during *in vitro* tests, or perhaps one of the factors in the process is supplied by diffusion through the teeth themselves. Further speculation concerning the cause and nature of the brown pellicle should probably wait until enough of the material has been collected to permit chemical studies that may show what salivary constituent the pellicle most nearly resembles.

SUMMARY

A brown pellicle has been found to form regularly on the teeth of some but not all of the persons who do not use dentifrice abrasives in their oral hygiene. The deposit can be readily removed by 1 or 2 brushings with dentifrice abrasives, and it will recur whenever abrasive brushings are omitted for 1 or 2 weeks. A method devised for examining anterior teeth with a binocular micro-

scope showed that the brown pellicle accumulated gradually and in a regular manner, and that repeated accumulations did not seem to affect the tooth surfaces in any way.

Under high magnification the deposit was seen to consist of a structureless, bacteria-free, faintly Gram-positive pellicle with which were frequently associated traces of a granular, Gram-negative substance. The pellicle gave positive protein tests and seemed to be chiefly organic material. It was insoluble in all of the chemical reagents tested at room temperature, and dissolved at 90°C. only in strong hydrolytic solutions, which suggested that it should be classed as a keratin. Some similarities between brown pellicle and known deposits were discussed.

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SOLUTION OF DENTIN POWDER BY INORGANIC ACIDS USED IN ROOT CANALS[1]

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Inorganic acids are used in root canals 1) to eliminate obstructions, such as pulp stones, adventitious dentin, broken instruments, etc.; 2) to disorganize or dissolve pulp tissue in the canal or pulp fragments adhering to the canal wall; 3) to overcome a curvature or constriction of the canal; and, most often 4) to widen or enlarge the root canal in order to gain access to the periapical tissues by way of the apical foramen, and to facilitate the filling of the canal. The disadvantages of using an acid are 1) possibility of irritating periapical tissue by diffusion of acid through apical foramen; 2) weakening of crown of tooth. The latter should rarely, if ever, occur but the author has seen a perforation of the pulpal floor of a mandibular molar tooth caused by prolonged use of an inorganic acid.

The use of an acid for enlarging root canals of teeth was probably first advocated by Callahan (1) in 1894. He recommended a 20% to 50% solution of sulphuric acid, describing its action as follows: "The solution softens the dentin for an indefinitely short distance; the broach removes this softened dentin, the acid instantly attacks the fresh surface, the instrument again cutting the softened surface, and so on, as long as the tooth is exposed to the action of the instrument and the acid." He stated that he had used the acid for almost 4 years. It is entirely conceivable that acids might have been used by others before the publication of Callahan's paper. According to Evans (2), sulphuric acid was used by Wm. H. Dwinelle in 1883 in conjunction with platinum broaches specifically made up for him. Whether he used the acid at the suggestion of Callahan is not stated.

Prior to about 1880 root canals were enlarged by means of small round burs or special broaches. Later, engine instruments were devised by J. G. Morey and by Gates for enlarging root canals. The latter instrument became the precursor of Gates-Glidden drills which are still being used to some extent for removing gutta percha in the root canal so as to prepare the canal for a post crown. Engine-driven instruments, however, never became popular because of occasional breakage in the root canal or because of perforation of the canal wall.

The introduction of sulphuric acid for enlarging root canals was followed soon

[1] Received for publication November 5, 1943.

The author is indebted to the Samson Laboratory for permission to use the Boerner shaking apparatus and other facilities for this study.

afterward by other acids, both organic and inorganic. Hays (13) suggested aqua regia in 1900 and Preiswerck (4) recommended hydrochloric acid. These clinical recommendations were purely empiric. On the basis of experimental work, Bruy (5) found aqua regia in reverse proportions the most effective of those acids examined by him, while sulphuric was the weakest. Montigny (6) confirmed Bruy's observations and, in addition, found 50% nitric acid the next most effective acid to aqua regia in reverse proportions. Sintenis (7) made a very thorough study of dentin solvents, examining most of the acids then currently employed by dentists for enlarging root canals. His method was to prepare 2 mm. blocks of dentin of sound teeth. These were dried in a drying chamber and weighed afterward on an analytical balance. Each dentin block was then placed in 1 cc. of acid for 15 minutes. The blocks of dentin were then washed, dried in the drying chamber, and weighed again. The amount of dentin lost indicated the effectiveness of the acid. He found that 50% reverse aqua regia solution was most effective and that solutions of 30% to 50% hydrochloric or nitric acid were next most effective. Peculiarly, he found that the dentin blocks exposed to sulphuric acid increased in weight rather than showed a weight loss. He conjectured that this was due to formation of calcium sulphate (plaster of Paris).

The present study was undertaken to determine the effectiveness of various acids which might be used as dentin solvents for enlarging root canals.

METHOD

Dentin powder was collected in the following manner. Extracted, sound human teeth were first washed in tap water then dried with a towel. Adherent bone, soft tissue and calculus were removed with revolving vulcanite burs. The tip of each tooth for a distance of 2 or 3 mm. was snipped off and discarded since it contained little available dentin. The cementum on the root surface was then removed by grinding with a large bur until dentin was clearly exposed. The exposed dentin was next ground with a clean, sharp #561 fissure bur revolving in a lathe at 1800 r.p.m. and the dentin powder was collected in a paper lined lathe pan. Not only the dentin from the root portion of the tooth, but also that from under the enamel cap was removed and collected in the manner described. The dentin powder was then successively put through 60 mesh, 100 mesh, and 200 mesh sieves. Only that dentin powder passing through a 100 mesh sieve but not passing through a 200 mesh sieve was used in this study. 30 mg. of this 100–200 mesh fine dentin powder was placed in a test tube and 2 cc. of acid was added. The test tubes were then shaken on a Boerner shaking machine for 10 minutes to facilitate maximum interaction between acid and dentin powder. The tubes were then centrifuged at 1500 r.p.m. for 2 minutes so as to separate undissolved dentin powder from acid. The process of shaking the tubes on the Boerner shaker and centrifuging was repeated until complete solution occurred (end point) or for a maximum shaking time of 130 minutes. All specimens were examined with a 4× lens.

The following acids were used in the tests: (Group 1) HCl, HNO_3 and H_2SO_4

each in 5/M, 2.5/M and 1/M concentrations. (Group 2) 50% aqua regia, 50% aqua regia in reverse proportions, and 50% HCl-HNO_3. (Group 3) Perchloric acid and phenolsulphonic acid. (Group 4) Concentrated (commercial) HCl, HNO_3, and H_2SO_4 were also tried, not because they could be used in clinical root canal practice but for the sake of completeness.

Each test was repeated a minimum of 5 times or a maximum of 20 times depending on how promising the acid appeared to be as a solvent of dentin.

RESULT

Fuming sulphuric acid, concentrated (commercial) hydrochloric acid and *concentrated nitric acid* effected complete solution of the dentin in from 5 to 10 minutes.

There was no marked difference in effectiveness between hydrochloric and nitric acid, although the latter was found to be slightly more effective. In general, the more concentrated the acid, the greater the solvent power, 5/M having been found more effective than 2.5/M and the latter more effective than 1/M. 5/M nitric acid effected complete solution in from 60–80 minutes, while 5/M hydrochloric acid effected complete solution in from 60–90 minutes. 2.5/M solutions of nitric acid or hydrochloric acid dissolved the dentin powder in 130 or more minutes, while 1/M nitric or hydrochloric acid did not effect solution in 130 minutes.

A 50% solution of *aqua regia in reverse* proportions dissolved the dentin powder in from 30 to 60 minutes while a 50% solution of *aqua regia* required from 60 to 90 minutes.

Perchloric acid was completely ineffective. A dense precipitate was formed with the dentin powder. *Sulphuric acid*, in all concentrations excepting the fuming acid, formed a precipitate with the dentin powder which was not soluble in the acid. This precipitate was apparently calcium sulphate formed by the interaction of sulphuric acid with the calcium carbonate of the dentin. *Phenolsulphonic acid* also formed a dense precipitate which prevented further solution of the dentin.

DISCUSSION

From a standpoint of clinical procedure, the concentrated commercial acids cannot be used even though they were found to be the most rapid solvents of dentin. A 50% solution of aqua regia in reverse proportions may be used clinically provided precautions are taken to confine the acid within the canal. It is preferably used with a stiff, non-corroding root canal instrument. A fine iridio-platinum needle, such as that used during electrolytic medication, may be employed to pump the acid into the root canal. Extreme care must be taken to confine the acid to the root canal. If a less active but nevertheless effective acid is desired for routine clinical treatment, one has a choice of 50% nitric-hydrochloric acid, 5/M (30%) nitric or 5/M (20%) hydrochloric acid. Inasmuch as sulphuric acid and phenolsulphonic acid form precipitates with dentin, they are less desirable for opening or enlarging root canals.

SUMMARY

(1) A method for determining the effectiveness of acids for dissolving dentin is described.

(2) From a clinical standpoint, 50% aqua regia in reverse proportions was more effective than any other acid examined which could safely be used in the root canal.

(3) Nitric acid was slightly more effective than hydrochloric acid.

(4) A 50% nitric-hydrochloric acid solution, or 30% nitric acid, or 20% hydrochloric acid may be used effectively for routine enlarging of root canals.

(5) Higher molar concentrations of either nitric or hydrochloric acid were more effective than lower concentrations.

(6) Sulphuric acid, phenolsulphonic acid and perchloric acid did not materially effect solution of dentin. Insoluble precipitates formed which prevented further solution of dentin.

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THE EFFECT OF MEDICAMENTS ON THE MOTILITY OF THE ORAL FLORA WITH SPECIAL REFERENCE TO THE TREATMENT OF VINCENT'S INFECTION. II.[1]

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A previous report (1) described an *in vitro* test of the antibacterial properties of medicaments intended for topical application to the oral mucosa. This method employs loss of motility of the motile oral flora and growth of the non-motile flora as a basis of comparison. The method has been adequately described and the apparatus illustrated in previous publications (1, 7). The motility test has certain advantages over the Rideal-Walker and similar tests, for it more nearly approximates clinical conditions. 1. It employs the actual bacteria which the drug is to combat, although some of these organisms cannot be grown in artificial media. 2. Since fresh saliva is used, the pH of the oral cavity, the organic and inorganic content, enzymes, putrefaction and fermentation products, detritus and end products of bacterial activity are preserved. 3. The ratio of medicament to saliva is closer to actual working conditions. 4. Chemical reactions on surfaces have been found to differ from reactions in bulk (2). While the motility test does not duplicate oral surfaces, it does have an enormously greater proportionate surface than the test tube. 5. Speed of action, power of penetration of debris, any specificity or coagulation (which would reduce penetration) are easily observed. 6. Results have closely corresponded to clinical results. 7. Loss of motility has proved to be loss of pathogenicity (3, 4).

This is a report on 27 additional medicaments which have been advocated, or may be useful, for the treatment of Vincent's infection.

THE SULFONAMIDES

Microcrystalline powders were employed to overcome the difficulty of obtaining an aqueous solution. The result was a suspension rather than a solution. No motility could be observed among the crystals, but motility was unaffected near the periphery of the crystalline mass. Subcultures were positive, although the number of colonies on the agar slants were markedly reduced. Sinclair (5) has reported unfavorable results following clinical use of sulfanilamide in Vincent's infection.

DRUGS WHICH REDUCE SURFACE TENSION

The most effective were *Zephiran*, pH 7.3, and *Tergitol*, pH 9.52. All motility ceased at the moment of contact and all subcultures were without growth.

[1] Received for publication October 25, 1943.

Amphyl (2%) and *Aerosol* (10%) likewise stopped motion on contact, but failed to reach many organisms because of poor power of penetration. Subcultures showed growth. *Teel* slowly penetrated organic material to stop all motility

TABLE I

Loss of motility

	SPIROCHETES	VIBRIOS	BACILLI	SUBCULTURES***		
				Hormone broth	Agar	Agar anaerobic
Sulfanilamide 15%†	15*	15	15	+	+	+
Sulfapyridine 15%	15	15	15	+	+	+
Sulfadiazine 15%	15	15	15	+	+	+
Sulfathiazole 15%	15	15	15	+	+	+
Chlorophyl	15	15	15	+	+	+
Kaodrox	15	15	15	+	+	+
Aludrox	15	15	15	+	+	+
Ascorbic Acid	Contact**	5	15	+	+	+
Viosterol (oil)	—	—	—	+	+	+
Zephiran 1:1000	Contact	Contact	Contact	0	0	0
Tergitol 1:10	Contact	Contact	Contact	0	0	0
1:100	2	2	3	0	0	0
Aerosol 10%	2	10	15	+	+	+
Teel	Contact	2	5	0	0	+
Amphyl 15%	15	15	15	+	+	+
Mallophene	15	15	15	+	+	+
Sobisminol sol	Contact	Contact	Contact	0	0	0
Tryparsamide	Contact	Contact	Contact	0	0	0
Sodium Carbonate 10%	Contact	Contact	Contact	0	0	0
Nutrox	Contact	Contact	2	+	+	+
Standard pectinate	5–15	5–15	5–15	+	+	+
Nickel Pectinate	Contact	Contact	Contact	+	+	+
Silver Pectinate	Contact	Contact	Contact	+	+	+
Bico	15	15	15	+	+	+
Xttrium	15	15	15	+	+	+
Negatan	Contact	Contact	Contact	0	0	0
Eugenol	Contact	Contact	Contact	0	0	0
Surgical Pack	—	—	—	Reduction		

* Figures represent the time required for loss of motility in minutes.

** Contact—instant loss of motility.

† Organisms still motile at the end of 15 minutes are marked 15.

All solutions are aqueous.

*** Non-motile organisms { + —growth; 0 —no growth

within 5 minutes. Curiously, subcultures grown anaerobically showed growth, although aerobic cultures were negative.

OTHER DRUGS

Chlorophyl was without effect.

Kaodrox and Aludrox. The claim is made that these suspensions act by

adsorption. This could not be substantiated. Obviously any drug used for topical application must act rapidly, since they will remain undisturbed for a limited time only. 15 minutes seems a reasonable limit for excluding saliva.

Direct applications of the vitamins have been advocated for treatment of gingival infections (6). A 50 mgm. tablet of *ascorbic acid* was dissolved in 15 cc. of distilled water. Spirochetes lost motility on contact, but the solution did not penetrate debris. Any slight amount of this material protected many organisms which continued motile. The apparent efficiency against spirochetes is explained by the pH 3.35. Any solution of like acidity will stop the movement of spirochetes. *Viosterol* was too viscid to test its effect on motility. Subcultures were all positive.

Mallophene, a dye, had little effect, nor did *Bico*. As previously noted (1), the spirocheticides (and particularly the organic arsenicals) are often efficient general bactericides. The efficiency of *Tryparsamide* and *Sobisminol* solution are further examples of this observation.

Sodium carbonate (*10%*) is an important antibacterial constituent of mammalian saliva. Details of its efficiency have been reported elsewhere (7, 8). *Nutrox*, a neutralized sodium perborate, pH 7.5 stopped motility of most organisms on contact. Its therapeutic action is not the result of sodium perborate. but the result of sodium carbonate formed from the interaction of the magnesium carbonate and sodium chloride included in the preparation. Tests of the ingredients and combinations thereof confirmed this fact. Clinical tests on 10 individuals did not reveal the usual chemical burns of sodium perborate.

Pectin was suggested for these tests because of its well known antibacterial properties. It is widely used as a preservative for jams and jellies. Standard *pectinate*, pH 3.6, *silver pectinate* containing 3.18% silver, and *nickle pectinate* containing 0.15% nickle were examined. The metallic pectinates were efficient against the motile organisms, but not against the non-motile flora.

The manufacturers of *Xttrium* claim remarkable efficiency for their product under the motility test. Attempts to duplicate such findings failed. Consultation with a company official revealed that the amounts of drug used were not controlled, and that the bacteria were inundated by huge quantities of liquid which swept away debris and bacteria alike. Actually Xttrium will accomplish no more than a Dakin's solution (9). The product has been unfavorably reviewed by the Council on Therapeutics (10).

Negatan, a highly acid solution, destroys all bacteria instantly by its highly caustic action. It coagulates tissue as well as bacteria, and has been employed to reduce hypertrophic gingivae (11). Such destruction is contraindicated in the treatment of Vincent's infection.

Zinc oxide-eugenol packs have gained much popularity in the treatment of gingival infections. These substances are the principal constituents of surgical cements. In order to determine their effect on the oral flora, 2 tests were made. The first showed eugenol to be highly efficient against the entire oral flora, motile and non-motile. To determine whether the eugenol is free after the cement is set agar plates inoculated with saliva were made. In the

center of each plate was a piece of a commercial surgical cement or a zinc oxide-eugenol mixture, all of which had been mixed 24 hours previously. After incubation, all plates had a large area of inhibition surrounding the cement. It can be concluded, therefore, that zinc-oxide-eugenol cements permit eugenol to act on the tissues.

SUMMARY

Twenty-seven additional drugs recommended for the treatment of Vincent's infection are evaluated (Table I).

Among the substances which are not injurious to the oral tissues, those instantly stopping movements of the entire oral flora and inhibiting growth of the non-motile flora are: Zephiran, Tergitol, Sobisminol, Tryparsamide, sodium carbonate (10%) and zinc-oxide-eugenol cements.

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FREQUENCY OF OCCURRENCE OF CLEFT PALATES AND HARELIPS[1]

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The frequency of the occurrence of cleft palates and harelips is apparently a matter of speculation since a review of the literature gives very few figures, and there is a wide variation in those that are given. In 1865 Froebelius reported 84 cases in 180,000 births or one to 2400. In 1867 Simon reported 17 cases in 18,846 births, 1 to 1109. In 1924 J. S. Davis (1) reported 24 cases in 28,085 births, 1 in 1170. Vaughan (2) stated that lip and palate clefts occur about once in every 1200 births.

Because we are developing a program within the Department of Health for the care of children having cleft palates, it seemed desirable to have some basis upon which to estimate the case load. A study was made of 202,501 birth certificates filed with the Bureau of Vital Statistics for the year 1942. It was found that in this group 250 children, born during the year 1942, were recorded as having cleft palates. This ratio, 1:800, seems to be higher than has been stated here-to-fore.

The figures are open to some criticism of inaccuracy. In the first place, the total number of births registered does not represent the total number of births which actually occurred. Lenhart (3) stated that in the entire United States for 1940, birth registration was only 92.5% complete, although in different sections of the country it varied. Pennsylvania was given as being 97% complete. The added emphasis placed upon the importance of birth registration by the war has probably assured an even better percentage for 1942. A review of the birth certificates showed a large number (3,569) to be incompletely or incorrectly filled out. These were subtracted from the total number for the purposes of this study, for we had no way of knowing whether these certificates recorded a normal baby or not. It is also recognized that some babies might have clefts of the palate which were not discovered until some time after birth because the lips were apparently normal and the oral cavities were not examined. In such cases the deformities were never recorded on the birth certificates. In spite of all the recognized errors in the compilation, it is still felt that the figures are sufficiently accurate to justify the statement that 1 child in every 800 born in Pennsylvania has some type of cleft palate, harelip, or both.

Cleft palates were reported from 51 of the 67 counties. The data reflected no particular geographical pattern. Philadelphia, which had 19.5% of the total State population in 1940, recorded 48 (19.2%) of the total of 250 cleft palates. Al-

[1] Received for publication Sept. 29, 1943.

legheny County, which had 14.3% of the total population, at the time of the last census, recorded 29 (11.6%) of the cleft palates. However, in comparing percentages of other counties it was found that they did not follow so closely the population density.

A study of the birth place of the mother showed nothing significant. Two hundred and six mothers were born in Pennsylvania, 9 were born in foreign countries, 2 of the birth places were unknown, and the other mothers were born in 14 sidely scattered states.

Length of pregnancy was also studied, but apparently little reliance can be placed upon this information as recorded on the birth certificates. Most of the records (206) gave 9 months.

The age of the mother at the time of the birth of the child is given in Table I. It has been stated by Blair and Ivy (5) that it is possible that the children born late in the reproductive age of mothers are more likely to have congenital defects than the first children born to these mothers unless there is a definite hereditary tendency to malformation. In this study, on the contrary, the highest percentage of cleft palates occurred in babies born by women in the age group 21–25, the most fertile age group.

TABLE I

Age of mothers at time of birth of cleft palate infants

AGE OF MOTHER	NO. HAVING CLEFT PALATE BABIES IN 1942	PER CENT OF TOTAL
All ages	250	100.0
15 yrs. to 20 yrs.	46	18.4
21–25	81	32.4
26–30	63	25.2
31–35	36	14.4
36–40	20	8.0
41–45	4	1.6

Of the 250 cleft palate babies born in 1942 a total of 104 (41.6%) were recorded as primiparous. Of all births in the United States in 1940, 35.8% were first born; in Pennsylvania in 1940 the figure was 38.6%. Later figures are not available. The following list shows the number of mothers of cleft palate infants with other children:

One other child	71
Two	28
Three	13
Four	11
Five	6
Six	5
Seven	5
Eight	2
Nine	1
Ten	1
Eleven	1

Whether any of these children also have this deformity we will attempt to find out later. This is of interest since insufficiency of inherent developmental force is given as an etiological factor in this deformity. Those who support this theory have stated that facial clefts are frequently found in families with large number of children.

Vaughan, Keith and W. B. Davis are of the opinion that this deformity is very rare among the negroes. This study showed that there were 241 white babies and 9 negro babies born with cleft palate. This is a lower ratio of colored to white than is shown in the total number of births. Of the total number born 5.5% were negroes, while only 3.6% of those born with cleft palates were negroes.

It would appear that more boys are born with cleft palates than girls, a fact which has been stated by many writers. The ratio, as usually given, is 105 boys born to 100 girls. However, our survey showed 155 boys to 88 girls. On 7 certificates, the sex was not recorded. Vaughan (3) from a series of 724 cases (409 boys and 315 girls) stated that boys are less subject to minor palate clefts but far more subject to the complete lip and palate clefts.

SUMMARY

A study of the birth records of 202,501 children born in Pennsylvania in 1942 showed that 1 in 800 had cleft palate. The county distribution did not suggest any significant pattern nor was study of the birth place of the mother fruitful. The highest percentage of cleft palate infants were born to mothers of the 21–25 age group. Of the 250 cleft palate infants 41.6% were primiparous while 38.6% of Pennsylvania babies (1940) were primiparous. Although 5.6% of the children born were negroes only 3.6% of the cleft palate infants were negroes. This group showed 155 cleft palate boys to 88 girls.

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[1] The proceedings of the twenty first general meeting (Chicago, March 13–14, 1943) were published in the *J. D. Res.*, 21: 191, 1943 (this volume).

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Section membership—504, listed in 31 sections. Average membership per section—16.

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Total Membership as of Dec. 1, 1943—604.

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[4] Members of the Materials Group may and in most instances belong to Sections.

III. BOOKS RECEIVED, 1943

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[1] Abbreviations used: I. A. D. R.—International Association for Dental Research; J. D. R.—Journal of Dental Research.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, R. G. Hutchinson, Jr.; T. P. Hyatt, Milo Hellman, A. H. Merritt, B. B. Palmer, P. R. Stillman, L. M. Waugh.

General Meetings have been held on this schedule: (1) New York, Dec. 21, '22. (2) Chicago, Mar. 7, '24. (3) Chicago, Mar. 20, '25. (4) Chicago, Mar. 26, '26. (5) New York, Apr. 19, '27. (6) Washington, Mar. 25–28, '28. (7) Chicago, Mar. 23–24, '29. (8) Toronto, Mar. 24–26, '30. (9) Memphis, Mar. 21–22, '31. (10) Columbus, Mar. 19–20, '32. (11) Chicago, Mar. 18–19, '33; adjourned executive session, New York, May 25, '33. (12) Chicago, Mar. 17–18, '34. (13) Chicago, Mar. 16–17, '35. (14) Louisville, Mar. 14–15, '36. (15) Baltimore, Mar. 13–14, '37. (16) Minneapolis, Mar. 12–13, '38. (17) Cleveland, Mar. 18–19, '39. (18) Philadelphia, Mar. 13–14, '40. (19) St. Louis, Mar. 15–16, '41. (20) New York, Mar. 14–15, '42. (21) Chicago, Mar. 13–15, '43.

Objects (*Const., Art. II*): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Dec. 1, 1942), 588. *Eligibility* (*Const., Art. 14, Sec. 1*).—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of March 10, 1939): $1,219.36. There have been no expenditures from this fund.

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Sectional meetings in 1942 were held on the following schedules as reported by sectional secretaries: *Boston*—March 2, April 21. *Chicago*—October 9, November 13, December 11. *Minnesota*—April 15, October 23. *New York*—January 29, May 28. *Pittsburgh*—January 5, May 13, October 5. *St. Louis*—February 9, December 17. *Washington*—January 28, April 14, November 17.

Journal of Dental Research: the Association's official publication. See register of officers and editors on page 2 of cover.

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The 22nd General Meeting of the International Association for Dental Research will be held at the Drake Hotel, Chicago, March 18–19, 1944; coordinated with the meetings of the American Association of Dental Schools, March 16–18, and Omicron Kappa Upsilon, March 17.

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